"EFFECT OF MUCUNA PRURIENS SEED EXTRACT ON TRI NITRO BENZENE SULPHONIC ACID (TNBS) INDUCED MODEL OF EXPERIMENTAL COLITIS IN MALE WISTAR RATS- AN EXPERIMENTAL STUDY".

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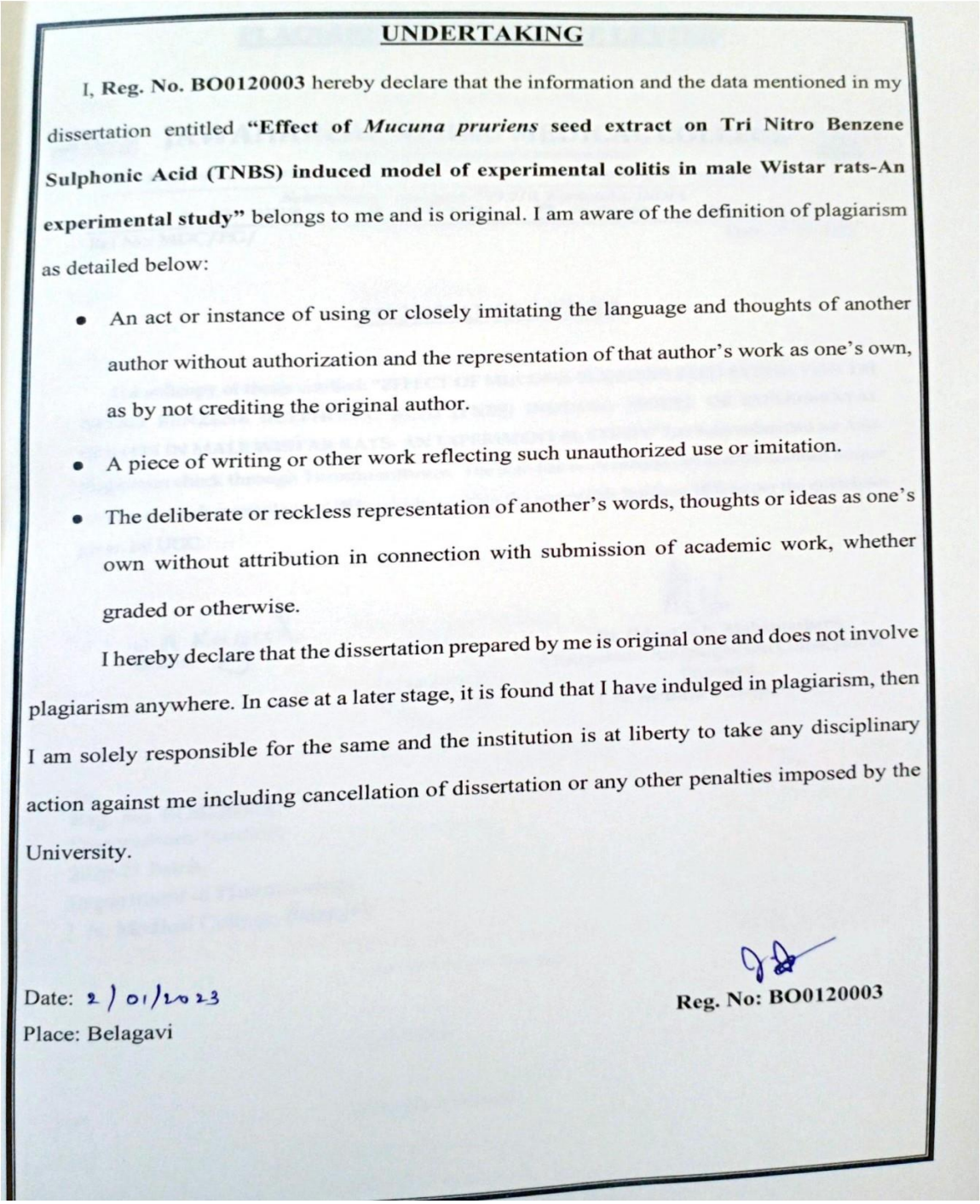

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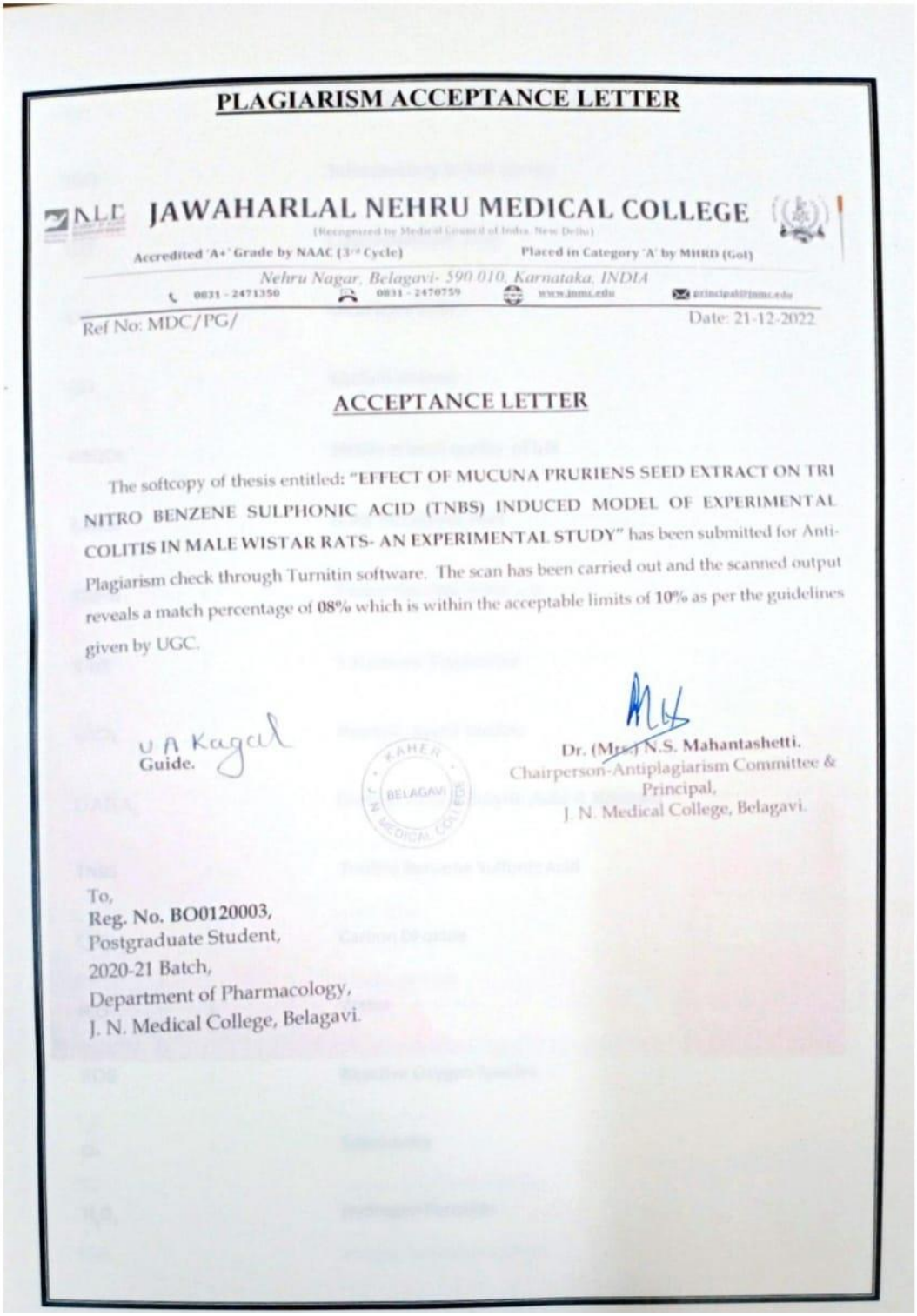

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LIST OF ABBREVIATIONS

IBD	:	Inflammatory bowel disease
GIT	:	Gastrointestinal Tract
UC	:	Ulcerative Colitis
CD	:	Crohn's disease
HRQOL	:	Health related quality of life
5-ASA	:	5-Aminosalicylic Acid
TNF-α	:	Tumor necrosis factor – α
5-HT	:	5-Hydroxy Tryptamine
nACh	:	Nicotinic Acetyl Choline
$GABA_A$	:	Gamma Amino Butyric Acid A Receptor
TNBS	:	Trinitro Benzene Sulfonic Acid
CO_2	:	Carbon Di-oxide
-OH	:	Hydroxyl radicle
H_2O	:	Water
ONOO-	:	Peroxynitrite
ROS	:	Reactive Oxygen Species
HOCl	:	Hypochlorous Acid
O_{2^-}	:	Superoxide
RNS	:	Reactive Nitrogen Species
H_2O_2	:	Hydrogen Peroxide
NO	:	Nitric Oxide
GSH	:	Glutathione

TBARS	:	Thiobarbituric acid reactive substances

Mp	:	Mucuna pruriens
NSAIDs	:	Non-steroidal anti-inflammatory drugs
CRP	:	C-reactive Protein
ESR	:	Erythrocyte sedimentation rate
CS	:	Corticosteroids
GC	:	Glucocorticoids
AZA	:	Azathioprine
6-MP	:	6-Mercaptopurine
6-TG	:	6-Thioguanine
TPMT	:	Thiopurine methyl transferase
MTX	:	Methotrexate

OCT 1	:	Organic cation transporter 1
IFNγ	:	Interferon gamma
VCAM	:	vascular cell adhesion molecule
KO	:	Gene Knockout
AICD	:	Acquired immune combined deficiency
MHC	:	Major histocompatibility complex
MCP-1	:	Monocyte Chemo Attractant Protein
GM-CSF	:	Granulocyte Macrophage–colony-stimulating factor
ARE	:	adenine/uracil-rich element
UTR	:	Untranslated Region
PG-PS	:	Peptidoglycan-Polysaccharide
STAT	:	Signal Transduction and Activator of Transcription
SH	:	Sulfhydryl
TCR	:	T-Cell Receptor
DSS	:	Dextran Sulphate Sodium
RCT	:	Randomized Controlled Trial
DNA	:	Deoxyribose Nucleic Acid
hsp	:	Heat Shock Protein
RNA	:	Ribonucleic Acid
KDa	:	Kilo Dalton
IAEC	:	Institutional animal ethics committee

Polymerase Chain Reaction

Real Time - Polymerase Chain Reaction

v/v	:	Volume by Volume
DAI	:	Disease Activity Index
ABI		Applied Biosystems

ABSTRACT

"Effect of *Mucuna pruriens* seed extract on Tri Nitro Benzene Sulphonic Acid (TNBS) induced model of experimental colitis in male Wistar rats- An experimental study."

Introduction & Objective:

Ulcerative colitis is a chronic inflammatory disorder involving the large intestine and rectum. However, the current therapy available is with limited efficacy and accompanied side effects. Thus, this study was conducted with the following objectives:

1. To determine the effect of *Mucuna pruriens* seed extract in Tri Nitro Benzene Sulfonic Acid (TNBS) model of experimental colitis in male Wistar rats.

2. To determine the effect of *Mucuna pruriens* seed extract on levels of inflammatory cytokines (TNF-α, IL-1β, IL-6)

Methods:

Experimental colitis was induced in Wistar rats using Tri-nitro benzene sulfonic acid (TNBS). Animals were divided into 4 groups with n=8 in each group. Induction of colitis was done using a modified infant feeding tube (6F) inserted per-rectally, under anaesthesia. Treatment was given with standard prednisolone (4.5mg/kg), test drugs, *Mucuna pruriens (*400mg /kg) once a day for 14 days. Weight measurement, food intake and Disease Activity Index was measured during the study period. At the end of study period rats were sacrificed and colon was dissected out. Half colon was used for gene expression study for TNF- α, IL-6 and IL-1b. Blood was collected for measuring oxidative stress. Remaining colon tissue was fixed in 10% formalin and sent for histopathological studies.

Results:

*Mucuna pruriens (*400mg /kg), effectively reduced the symptoms of colitis and pro-inflammatory cytokine levels in TNBS induced colitis in Wistar rats.

Conclusion:

This present study proved significant anti-colitis effect of *Mucuna pruriens.* Based on these finding and the favourable safety profile of this herbal drug, it is recommended to plan and conduct further studies and clinical trials to expedite their introduction into clinical practice.

Keywords: Ulcerative Colitis, TNBS, *Mucuna pruriens*

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INTRODUCTION

In nature, everything is all about balance. It maintains the quality of life and easy functioning of this universe. The process of inflammation is responsible for maintaining the balance of human body. Inflammation shields our body from outside invading agents such as microbes and injuries. This inflammatory response concentrates and eliminates to foreign particles, microorganisms and antigens that opens the way to normal structure and function.[1] Any imbalance to these inflammatory mechanisms causes diseased states.

Inflammation can be acute or it can proliferate and turn to chronic inflammation. Chronic inflammation can either follow acute inflammation or it can be sudden in onset. Acute inflammation has several steps namely initiation, amplification, destruction and termination of inflammation, once the offending agent is no longer exists. It has also been observed that particular types of injuries to the body in susceptible individual, triggers the immune and inflammatory responses leading to chronic inflammation.[2] In chronic inflammation, inflammatory cells are permanent due to tissue destruction. The stroma becomes hyperplastic and scarring may lead to organ dysfunction.[1] This process may be limited, but it progresses to diseases such as Ulcerative Colitis, chronic dermatitis, granulomatous diseases, rheumatoid arthritis, autoimmune diseases and chronic lung disease.[1,2]

IBD is characterised by persistent or recurrent immunological activation and intestinal inflammation (GIT). Crohn's Disease (CD) & Ulcerative Colitis (UC) are the two major types in IBD , that having more clinical & epidemiological similarities that suggest a underlying aetiology.[3]

IBD is predominantly observed in western countries with current estimated number of approximately 800000 cases with Ulcerative Colitis in US and 1.4 million cases in European countries.[3,4] A latest systematic review declares that the prevalence and incidence of Ulcerative Colitis is increasing at the global level .[5] Currently ,there is a change in the lifestyle modifications in Asian countries, the prevalence and incidence of ulcerative colitis is on the peak and prevalence rates of ulcerative colitis in Asian countries like India are comparable to people from Western countries.[3,6]

In India, Ulcerative Colitis had been considered a unique disease. But a study which was conducted in North India in the year 1965, showed among 79 patients presenting with chronic diarrhoea, 69 patients were reported to be affected from non-specific ulcerative colitis.[8] There exists poor availability of data in prevalence & incidence of the disease. In India, population-based study in Punjab showed a prevalence of 44.3 per lakh and an incidence of 6.02 per lakh population.[7,8]

The increasing prevalence in Asia is possibly related to increasing development of industrialisation and is in connection to increased diagnostic accuracy due to the emergence of modern technology. If the similar trend continues in Asia, the prevalence rates are likely reach the rates similar to the west. Ulcerative Colitis in India is found to have a North – South gradient with more disease progression being recorded from North India.[8] At present, India has the highest incidence of IBD among the Asian countries [7,8]

IBD, clinically it is markedly represented by episodic exacerbations and remissions. The mainstay of treatment for inflammatory bowel disease is to alleviate the episodes of exacerbations and to maintain the remission in patients. As the disease is inoperable, it requires lifelong treatment. There is a significant reduction in the health-related quality of life (HRQOL) of such patients. [10]

In Ulcerative Colitis, conventional drugs are used namely, corticosteroids, antibiotics, amino salicylates and immunomodulators. The most commonly used regimen incorporates 5-Aminosalicylic Acid (5-ASA) for maintaining remission and corticosteroids during acute episodes. 30 percent of patients, who are receiving amino salicylates, present with the side-effects like nausea, vomiting, headache, fever, rashes, agranulocytosis, hepatitis, pancreatitis, nephritis, and male infertility. Short-term use of steroids having the side-effects which includes, mood swings, weight gain and fluid retention. Long-term use of steroids increases osteoporosis, risk of cataracts, myopathy and adrenal insufficiency along with immune- suppression.[9,11]

The above-mentioned drugs are unable to arrest the pathology of inflammatory bowel disease or used in the treatment of its complete remission. Their adverse side-effect profile possesses harmful to patients receiving these drugs life-long. Therefore, more scientific

research is required to develop safe and effective medications to treat inflammatory bowel disease, particularly Ulcerative Colitis.

Many drugs are tested for the management of Ulcerative Colitis with varying degrees of success. These includes Tumour Necrosis Factor α (TNF-α) antagonists like Adalimumab, Golimumab and Infliximab [12,13], and anti-integrin agent like Vodolizumab [12,14] and sphingosine-1-phosphate receptor agonist Ozanimod[15]. The biological agents have proven to be quite effective, but they are associated with great economic burden to the patient and have many adverse effects .[16,17]

Mucuna pruriens belonging to the Fabaceae family, is commonly known as (Velvet bean) cowage plant / kapikacho and is a popular Ayurvedic and Unani medicine. *Mucuna pruriens* is known to possess anti-inflammatory property. Phytochemicals in the *Mucuna pruriens* seed extract are involved in molecular mechanisms which results in modulating inflammation related cellular function. *Mucuna pruriens* possesses no or minimal side effects.[18]

From the current literature, it can be concluded that more scientific research is required to develop safe and effective medications to treat inflammatory bowel disease, particularly Ulcerative Colitis. Also, many drugs have been tested for the management of Ulcerative Colitis.

Mucuna pruriens has been found to have anti-inflammatory property and has been associated with minimal or no adverse effects. Hence this study, was planned to estimate the outcome of *Mucuna pruriens* Seed extract on Trinitrobenzene Sulfonic Acid (TNBS) induced experimental colitis in male Wistar rats.

OBJECTIVES OF STUDY

- **Primary Objective:**

  To evaluate the effect of *Mucuna pruriens* seed extract on Tri-Nitro Benzene sulfonic Acid (TNBS) model of experimental Colitis in male Wistar rats.

- **Secondary Objective:**

  To evaluate the effect of *Mucuna pruriens* seed extract on levels of inflammatory Cytokines like (TNF- α, I L- 6, IL-1 β) on Tri-Nitro Benzene sulfonic Acid (TNBS) model of experimental Colitis in male Wistar rats.

REVIEW OF LITERATURE

1. Introduction- History
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    - 9.5 Less frequently used model.

INTRODUCTION:

- Inflammatory bowel disease (IBD) is a chronic, idiopathic and relapsing inflammatory condition mostly invading the small intestine and colon and it is characterized by intense pain in the abdomen and diarrhoea. IBD majorly includes Ulcerative Colitis (UC) and Crohn's Disease (CD). [19]
- It is a chronic multifactorial inflammatory disorder characterized by periods of activation and reoccurrence of intestinal inflammation, with potentially severe complications, that can lead to death.[20]
- For understanding the pathogenesis of major IBD phenotypes in humans, that is Crohn's disease (CD) and ulcerative colitis (UC), experimental animal models of intestinal inflammation are much needed.[21]

• Animal models have crucial role on revealing with the molecular framework of IBD & while no model could express the diversity of disease, they have all made a significant contribution to our knowledge of its various aspects.[19]

- Tri-nitro benzene sulfonic acid (TNBS)-Induced Colitis, a Chemically-induced model in inflammation of intestine is widely used. [22]

HISTORY

- UC was first described by two English physicians. Wilks and Moxon in 1875, who distinguished it from diarrhoeal diseases caused by bacterial and viral agents.[23]
- There is evidence with symptoms of disease same as Ulcerative Colitis in the 16th and 17th century but it was not termed as a unique disease until 1870. In 1932 Crohn's Disease was described by three doctors— Gordon D. Oppenheimer, Leon Ginzberg, Burrill Crohn.[24]

- In the late 18th and early 19th centuries, intestinal TB was the diagnosis for any condition that have impact on colon & small intestine.[25] The doctors recruited primary data from 14 patients with fever, cramps in abdomen, diarrhoea, weight loss and identified that symptoms are not caused by intestinal TB or other diseases. As a result, they found new disease entity called regional ileitis, which is known as Crohn's disease.[26]

PATHOPHYSIOLOGY OF INFLAMMATORY BOWEL DISEASE

- Although the exact etiology of IBD is unknown, studies on its molecular mechanisms during chronic development of disease reveals changes in the gut microbiota, immune system and mucosal barrier, as well as elevated levels of oxidative stress and pro-inflammatory Cytokines.[27]

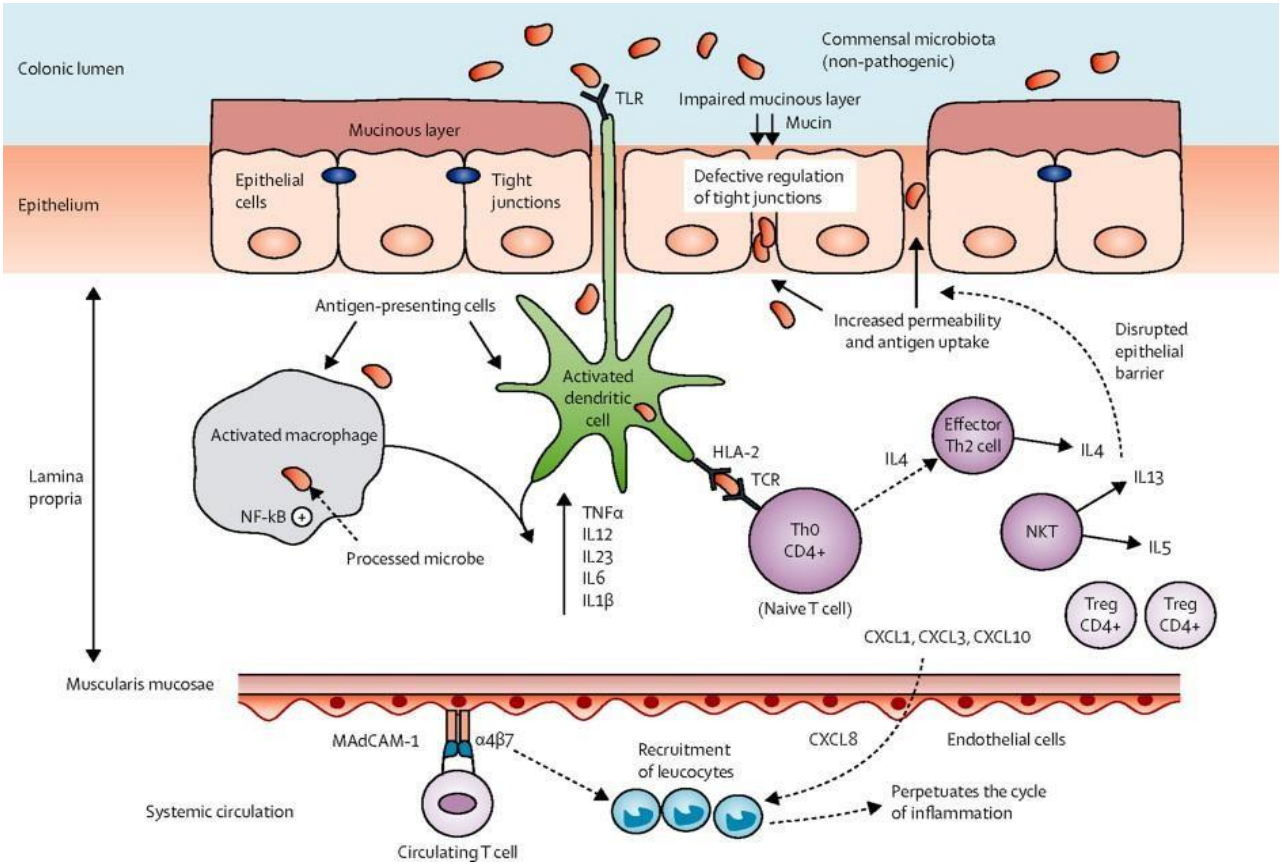


FIGURE 1: SCHEMATIC DIAGRAM SHOWING THE PATHOPHYSIOLOGY OF IBD: IMMUNE RESPONSE.[28]

GENETIC PREDISPOSITION IN ULCERATIVE COLITIS

- Inflammatory bowel disease is a familial disease and has incidence of 5–10% of patients.
- There have been great advances in recent times in the complex genetics of inflammatory bowel disease. Studies were done based on single nucleotide polymorphisms and candidate gene approaches and also on transgenic and deletion techniques.[28]
- It is thought that Ulcerative colitis and Crohn's disease are heterogeneous polygenic disorders, involving some but not all susceptible loci with several factors determining the disease phenotype.
- Presence of mutated gene does not ensure that inflammatory bowel disease will develop.[29]

Studies have shown that concordance rates for inflammatory bowel disease are much lower in ulcerative colitis in comparison to Crohn's disease, indicating that genetic penetration is much less in ulcerative colitis.[28] Reported concurrence rate for ulcerative colitis in monozygotic twins is 15.4% vs. 3.9% in dizygotic twins.[30]

INNATE AND ADAPTIVE IMMUNITY

The immune system is the collection of cells and macromolecules which safeguards the body from numerous pathogenic microbes and toxins in the environment.
This protection against microbes involves two types of reactions:

- reactions of innate immunity and
- reactions of adaptive immunity.[31]

- Innate and adaptive immunity are two evenly important views of the immune system in which innate immune response works as a first line of defence and provides nonspecific protection whereas adaptive immune response is highly distinct in nature and gets activated by the innate immune system.[32]

- ❖ Innate immunity is made by the epithelial barrier, neutrophils, macrophages, dendritic cells and natural killer cells (NK cells).[33] It is triggered by toxins and microbial agents which are identified by the specific recognition receptors, like toll-like receptors (TLRs) and NOD-like receptors.[34]
- ❖ Neutrophils along with other cells start the process of inflammation by liberating proinflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-8, which triggers the adaptive immune response.[35]

- Adaptive immune system consists of T-lymphocytes and B-cells, which on activation releases cytokines and antibodies. T cells are the main donor to inflammatory bowel disease due to the increased production and release of proinflammatory cytokines and interferons (IFNs) in the alimentary tract. IL-12 triggers the Th1 cells to produce huge number of IFNs and mainly responsible for inflammation in Crohn's disease patients.[36] Th2 cells increase the production and release of IL-4, IL-5, and IL-13 and are responsible for inflammation in ulcerative colitis patients.

Th3 cells has been involved in inflammatory bowel disease pathogenesis (Th17 cells) cells causing the release of IL-17 and IL-22 and play an important role in the damage of local tissue.[37]

- Proinflammatory cytokines damages the epithelial barrier and increases gut permeability; thereby it leads to an uncontrolled inflammation.[38]

ROLE OF INTESTINAL MICROBIOTA

- The damage in gut bacterial flora is well described in the pathophysiology of inflammatory bowel disease. Modification in the intestinal mucosa layer in inflammatory bowel disease results in increased connection of gut bacteria to innermost lining of mucosa.[39] This association of bacteria is identified as hapten and results in activation of the inflammatory process by binding on antigen-presenting cells (APC) and this ultimately causes the damage to mucosal layer.[40]

Though the clear role of gut bacterial flora in the pathogenesis of inflammatory bowel disease is not clear, but some of investigators have demonstrated that some bacterial species like *Bacteroides* or *Clostridium* spp. and aerobic E. increased in inflammatory bowel disease.[41]

DISRUPTION OF MUCOSAL BARRIER

- Mucosal surface consists of epithelial cells and it forms a barrier between hostile external environments and the internal milieu.[42] Mucosal surfaces have selective permeability barrier and are responsible for nutrient absorption and waste secretion.[43] Variations of the intestinal mucus layer concerning its thickness, continuity and composition, as well as the mucin-structure have been reported in inflammatory bowel disease patients, especially in Ulcerative Colitis.[44]

- These changes are thought to adversely affect the protective properties of the gel layer and consequently causes an increased bacterial invasion to the epithelial layer. Damage of this barrier results in disruption of gut epithelial permeability, which can induce an overactive mucosal immune response and chronic intestinal inflammation. The exact mechanism of the loss of barrier is not known, but several studies have reported that cytokines, TNF-α, interleukins are involved in the pathogenesis of Ulcerative Colitis and Crohn's Disease that leads to the increased epithelial permeability and alteration of tight junctions causing the barrier disruption.[45]

INFLAMMATORY MEDIATORS:

- Inflammatory bowel disease is a chronic inflammatory condition which involves genetic, environmental, and biological factors trigger inappropriate immune responses in which there is an overproduction of different proinflammatory mediators, such as TNF-α, ILs, cytokines, and chemokines.[46]
- The concentration of these mediators is highly elevated in blood, stool, and intestinal mucosa of IBD patients.[47] Inflammatory mediator profiles of Crohn's disease and ulcerative colitis are different. IL-2 and IFN-γ are responsible for the inflammation in CD whereas IL-4, IL-5, and IL-10 are involved in ulcerative colitis.[48]

- The release of these inflammatory cells is regulated by different pathways involved in inflammation, namely nuclear factor-kB (NF-kB), Mitogen-Activated Protein Kinases (MAPK) pathway and JAK/STAT pathway. NF-kB and MAPK are activated by the TLR-4 which stimulates the release of TNF-α, IL-6, and IL-12 through macrophages and enhances the inflammatory response. In addition, cytokines exert their signalling by activating JAK/STAT pathway and are involved in the production of a number of interleukins. The proinflammatory mediator release is the major factor involved in the progression of inflammatory bowel disease.[49]

ENVIRONMENTAL FACTORS

Epidemiological studies have shown that prevalence of inflammatory bowel disease is dramatically increased in countries where there is more "westernization" of lifestyle.[50]

- Westernization of lifestyle, includes environmental triggers such as smoking (shown to be protective in ulcerative colitis but detrimental in Crohn's disease), use of antibiotics and nonsteroidal anti- inflammatory drugs (NSAIDs), stress, infection and diet.
- A study has also reported an association between early life exposure to antibiotics (in the first year of person's life) and Crohn's disease development due to early childhood dysbiosis.
- The mechanisms of environmental influences are unclear. Studies have shown some evidence that infection and NSAIDs can transiently initiate nonspecific inflammation, disrupt the mucosal barrier and activate innate immune response.[51]
- There are also studies done which show that appendectomy and smoking reduce the risk of ulcerative colitis but on the other hand, active smoking increases risk for Crohn's disease.
- According to "Hygiene Hypothesis" lifestyle changes which lead to low microbial exposure due to improved living conditions and higher utilization of antibiotics there has been "westernization" of the population leading to increased prevalence of IBD.[52]

- Even though there are many epidemiological studies and evidence, linking IBD to certain environmental factors, it is still widely believed that there is no single environmental factor that could alone cause inflammatory bowel disease.[53]
- Previous appendectomy with confirmed appendicitis, breast feeding, and diets high in animal protein, sugar, sweets, oils, fish and dietary fat have shown a protective effect in IBD.[54]
- It has been seen that females using oral contraceptive pills and children using antibiotics have increased risk of IBD.[55]

On 19th May every year, "World IBD day" is celebrated to increase the awareness to fight against the disease.

FIGURE 2 : The purple ribbon

(https://worldibdday.org/).

(Symbol of awareness and support subject's surviving with inflammatory bowel disease)[56]

PATHOLOGY OF ULCERATIVE COLITIS

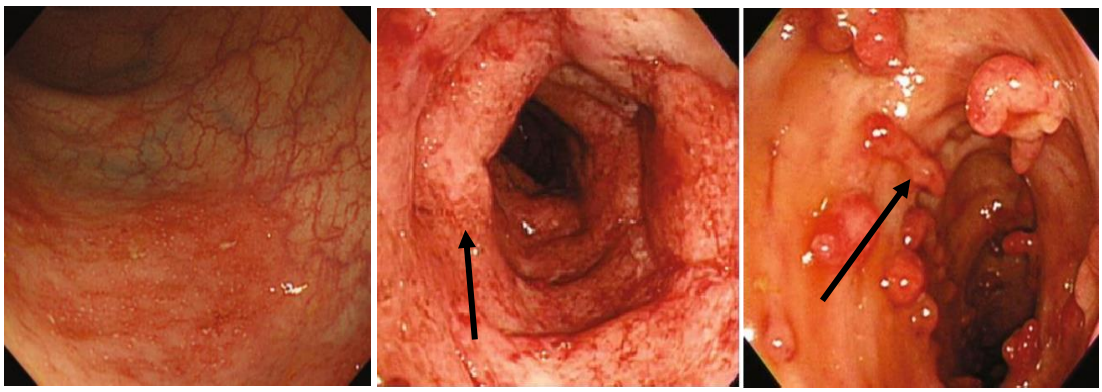
MACROSCOPIC PRESENTATION

- Ulcerative colitis is a mucosal disease that extends from the rectum to all or part of the colon.

In about 50% individuals there is involvement of the rectum only, 30% shows involvement of rectum and some part of the colon and about 20% have entire colitis.[57]

- In mild inflammation, there is an erythematous mucosa and has a fine granular surface that represents the sandpaper appearance. In more severe disease, the mucosa is haemorrhagic, oedematous, and ulcerated. In long standing cases, there is a presence of inflammatory polyps.[58]

- In fulminant stage, toxic colitis or megacolon develops which leads to perforation.



(a) (b) (c)

FIGURE 3: COLONOSCOPY IN ULCERATIVE COLITIS

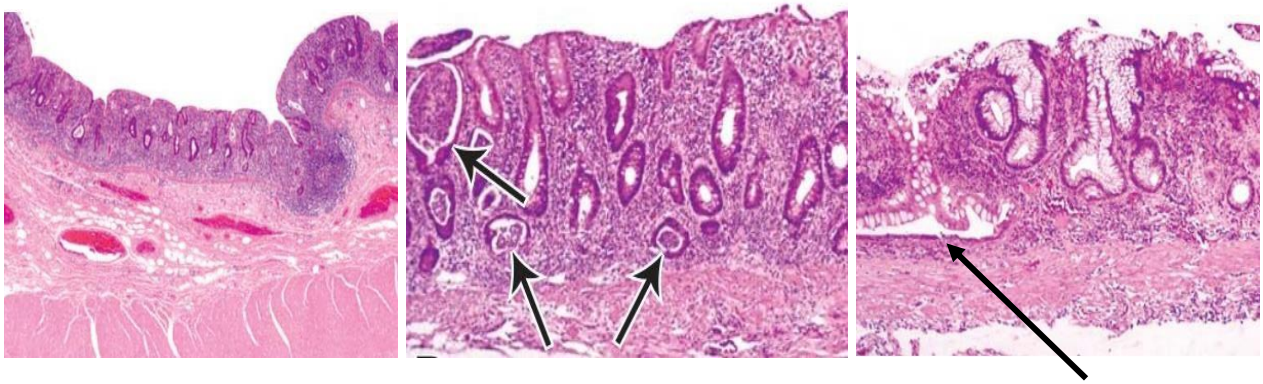
(a) Mild, (b) Haemorrhagic, (c) Inflammatory polyps.[59]

MICROSCOPIC PRESENTATION

The histology findings correlate to the clinical course and colonoscopy findings. This process is limited to the mucosa and superficial submucosa. The histological findings depict mucosal congestion, oedema and tiny haemorrhages. Damage and distortion of colorectal crypts, which are often surrounded and infiltrated by neutrophils is also evident from histology.[60]

First, there is a distortion in the architecture of the crypts of the colon; crypts may be branched and reduced in number, more often with a gap between the crypt bases and the muscularis mucosae. In late or advanced ulcerative colitis, mucosal atrophy and chronic inflammation are located in the mucosa and superficial submucosa. The most common is Paneth cell metaplasia .[61]

FIGURE 4: MICROSCOPIC VIEW IN ULCERATIVE COLITIS.[59]



Crypt distortion Crypt distortion and atrophy

(a) (b) (c)

(a) full thickness view showing mucosal involvement only

(b) arrows showing crypt abscesses and distortion

(c) Chronic UC with significant crypt distortion and atrophy.[59]

CLINICAL PRESENTATION:

- Ulcerative colitis is represented by stages of exacerbations and remissions. The major symptoms are diarrhoea, rectal bleeding, tenesmus, passage of mucous and crampy abdominal pain.
- Symptoms correlates with severity of disease. When there is only rectal involvement, passage of fresh blood or blood mixed mucous and tenesmus with feeling of incomplete evacuation but distally only constipation is presented.
- As the disease extends beyond rectum, blood with stool or gross bloody diarrhoea is seen. In severe disease, there is liquid stools with blood, pus and faecal matter.[62]

DIAGNOSIS OF ULCERATIVE COLITIS:

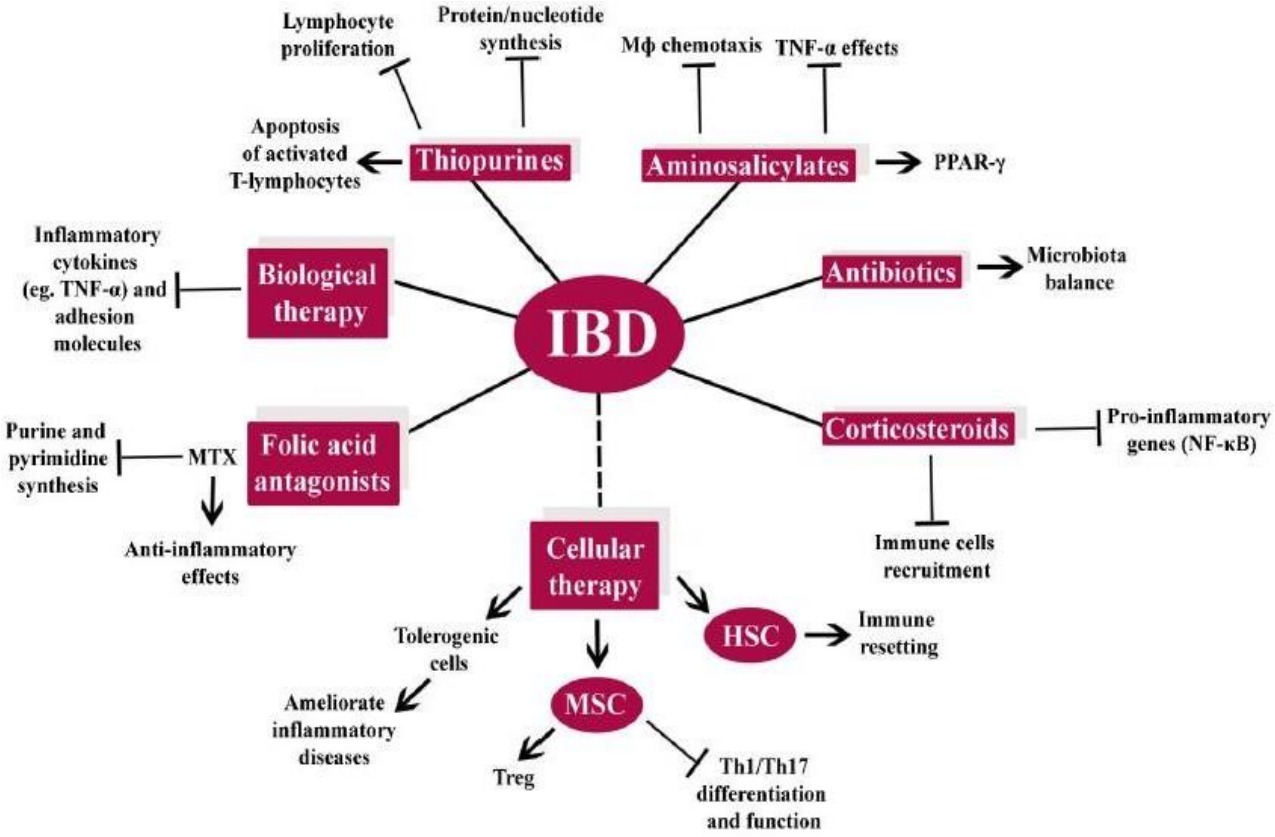
- Ulcerative colitis is diagnosed by history of the patient and clinical findings and confirmation can be done by colonoscopy with collection of tissue for biopsy.[63]
- Active disease is associated with elevated C-reactive protein (CRP), platelet count, erythrocyte sedimentation rate (ESR) and a decrease in haemoglobin count.[64]
- Faecal lactoferrin is highly sensitive to detect intestinal inflammation. Faecal calprotectin levels correlate well with inflammation and relapses can be predicted to detect pouchitis. In severely ill patients there is a rapid fall in albumin levels.
- Diagnosis relies on the patient's history; clinical symptoms; negative stool examination for bacteria, *C. difficile* toxin, and ova and parasites; sigmoidoscopic appearance; and histology of rectal or colonic biopsy specimens.[65]

PHARMACOTHERAPY OF INFLAMMATORY BOWEL DISEASE

Medical Management of Ulcerative colitis

- Current available therapy is targeted towards the control of the inflammatory condition; however, there is lack of monotherapy to control the condition. Specific goals of pharmacotherapy in inflammatory bowel disease include controlling acute exacerbations of the disease, maintaining remission and treating specific complications.[66]
- The mainstay of treatment is glucocorticoids, sulfasalazine and other 5-ASA agents. More recently immunomodulators like azathioprine and cyclosporine, which are routinely employed in other immune or inflammatory conditions, have been adopted for inflammatory bowel disease therapy.[67]

FIGURE 5: PICTURE SHOWING THE CURRENT AND EMERGING MEDICAL THERAPIES FOR IBD[68].



- In mild to moderate ulcerative colitis, 5-ASA are used and to maintain remission after symptoms subsides.[70] The glucocorticoids as inhibitors of neutrophil formation and activation, capable of decreasing the margination of neutrophils, and inhibiting neutrophil aggregation and eicosanoid metabolism are indicated primarily for induction of remission and long-term-maintenance.[71]

DRUGS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES

6.1. MESALAMINE THERAPIES

Drugs in this class are:

- Sulfasalazine, Mesalamine, Balsalazide and Olsalazine.

  Sulfasalazine (prototype drug, serendipity drug), it was discovered accidentally for the treatment of inflammatory bowel disease when it was used as a trial drug for the treatment of rheumatic polyarthritis.[72]

- It was revealed that the active moiety of the compound was 5-aminosalicylic acid (5-ASA) and that the sulfapyridine moiety acted as a carrier after 35 years ago, thus paving the way for the development of second generation of compounds. Since then, 5-ASA (Mesalamine), not only used for the treatment of Inflammatory but also believed to possess the chemo-protective properties, therefore reducing the risk of developing colorectal cancer.[73]

- Sulfasalazine is absorbed in the small intestine and excreted as bile. The therapeutically inactive carrier, sulfapyridine, are formed from the reductive cleavage of the azo bond by bacterial azoreductase, undergoes metabolism within the colonic lumen.[74]

- The theories of several mechanisms are as follows:
- It has been suggested that, mesalamine modulates the specific humoral inflammatory responses such as blocking the production of leukotrienes and prostaglandins.
- Several extended theories describes that the inhibition of leukocyte chemotaxis and scavenging of oxygen-derived radicals.
- Mesalamine inhibits a major inflammatory signal, tumour necrosis factor (TNF)-dependent nuclear factor kappa B (NF-κB)[75]
- 5-ASA (mesalamine) promotes peroxisome proliferation activated receptor-gamma (PPAR- γ), thereby interfering with the Nuclear Factor kappa beta NF-kB pathway.[76]
- Different formulations which release mesalamine at different sites are taken into consideration for disease at different sites in alimentary tract.
- These formulations include controlled release preparations (Pentasa®), pH dependent coating and multi-matrix core system (lialda®), pH dependent resin (asacol®), granulated mesalamine with pH dependent coating with extended-release polymer matrix core (salofalk®)[77] and in form of prodrug which take advantage of bacterial azoreduction to release 5-ASA in the colon, which include sulfasalazine. Mesalamine is also given as rectal suppositories and enemas.[78]

- 5-ASAs are having few serious adverse effects.

  Sulfasalazine, due to its sulfapyridine moiety causes adverse effects, mild side effects include fever, rash, headache, dyspepsia, diarrhoea. Rare serious adverse reactions to sulfasalazine are hepatitis, pancreatitis, leukopenia, haemolytic anaemia, neurotoxicity, and pulmonary fibrosis.[79]

- Serious adverse effects with Mesalamine include blood dyscrasias, pancreatitis, and nephrotoxicity. The maintenance dose for the treatment of active colitis of mesalamine ranges between 1.2 to 4.8 g/day.[17]
- Sulfasalazine dose ranges between 2 to 4 g for active and quiescent disease, but the side effects are frequently seen above 2g/day in dose dependent cases.

TABLE 1: MESALAMINE-BASED DRUGS FOR THE TREATMENT OF ULCERATIVE COLITIS.[80]

DRUGS	THERAPEUTIC USES	CLINICAL PHARMACOLOGY
MESALAMINE-BASED DRUGS		
Mesalamine (5-ASA)	Used in the treatment of mild-to-moderate ulcerative colitis	• On oral administration, jejunum is primary site of absorption, therefore the effect in more distal disease is limited. • It can be delivered as a rectal suppository in rectal conditions.
Sulfasalazine	Used in the treatment of mild-to-moderate ulcerative colitis For severe ulcerative colitis, it is used in combination with glucocorticoids	Sulfasalazine is a prodrug, that delivers 5-ASA to more distal GI parts following metabolism by colonic bacteria. • Sulfapyridine is also released; It causes adverse effects in patients sensitive to sulfa-drugs.
Olsalazine	Used in the treatment of mild-to-moderate ulcerative colitis.	• Olsalazine is a prodrug with two azo-linked 5-ASA molecules

	For severe ulcerative colitis, it is used in combination with glucocorticoids	• It eliminates the side effects associated with the sulfapyridine moiety of sulfasalazine
Balsalazide	Used in the treatment of mild-to-moderate ulcerative colitis. For severe ulcerative colitis, it is used in combination with glucocorticoids	Balsalazide is a prodrug with a 5-ASA molecule linked with an inert, unabsorbable second moiety molecule. • It eliminates the side effects associated with the sulfapyridine moiety of sulfasalazine

6.2. Corticosteroids

- About 60 years ago, Corticosteroids were first suggested by Truelove and Witts. Corticosteroids are the drugs which are successfully used for the treatment of inflammatory bowel disease.
- There has been a tremendous struggle in the application of corticosteroids in inflammatory bowel disease. When the medications are given systemically for long periods of time their significant adverse effects occur. In current use, both systemic and topical application therapies are available.
- The Current American Gastroenterological Association guidelines recommends that the patients who do not respond adequately or are intolerant to first-line therapy with 5-ASA formulations with moderate to severe ulcerative colitis can use conventional corticosteroids, such as prednisone, in mild to moderately active ulcerative colitis.
- Induction of remission can be effectively achieved with prednisone administered at doses 40–60 mg/day (or 1 mg/kg/day) with an average of 7–14 days.
- The dose of prednisone should be tapered by 5 mg/week to a dose of 20 mg once remission is achieved.

- Patients failing to respond to oral Corticosteroids or presenting with severe ulcerative colitis may benefit from parenteral corticosteroids (40–60 mg/day of methylprednisolone or 200–300 mg/day of hydrocortisone).
- Topical therapeutic agents, like hydrocortisone or budesonide are administered rectally for the treatment of ulcerative colitis located in distal part of colon.
- Topical Corticosteroids, such as budesonide (newer generation) given rectally at doses between 2 and 2.5 mg, were found to be equally effective as conventional Corticosteroids administered at doses between 100 and 125 mg of hydrocortisone equivalent and administered rectally in producing symptomatic, endoscopic and histologic remission.[81]

TABLE 2: GLUCOCORTICOIDS FOR THE TREATMENT OF ULCERATIVE COLITIS.[83]

Glucocorticoids: Minimize duration of use. Taper dose prior to topping to minimize disease relapse and avoid adrenal insufficiency.		
DRUGS	**THERAPEUTIC USES**	**CLINICAL PHARMACOLOGY**
Prednisone	Used in the treatment of moderate-to-severe Crohn's disease and ulcerative colitis.	Prednisone -Hepatic metabolism to active moiety. It is not used for maintenance therapy because of serious adverse effects
Methylprednisolone	Used in the treatment of moderate-to-severe Crohn's disease and ulcerative colitis.	When it is administered orally, intravenously, or intramuscularly. Patients respond poorly to oral prednisone. It has higher incidence of Na+ retention and K+ wasting. So, it is preferred over hydrocortisone.

Hydrocortisone	Used in the treatment of moderate-to-severe Crohn's disease and ulcerative colitis.	Patients who respond poorly to oral prednisone Hydrocortisone is administered intravenously.
Budesonide	Used in the treatment of moderate-to-severe Crohn's disease and ulcerative colitis.	It has prominent first-pass metabolism that reduces side effects and can result from higher maintenance of systemic levels.

6.3. IMMUNOMODULATORS

Immunomodulator-class medications include the following:

• Methotrexate, 6-mercaptopurine, and azathioprine. Other medications include tacrolimus, thalidomide, mycophenolate mofetil, cyclosporine A, and 6-thioguanine.

• Immunomodulators are the substances that aid in preserving the remission state; however, they have been found to be more harmful than 5-ASAs and glucocorticoids.[84]

- **AZATHIOPRINE / 6-MERCAPTOPURINE:**
- AZA and 6-MP are purine analogues that have a variety of effects on the immune system, including the reduction of DNA and RNA production, which reduces natural killer (NK) T cells over a period of weeks to months and inhibits cell-mediated immunity. By interacting with the enzyme Rac1, activating target genes such nuclear factor-B and mitogen-activated protein kinase, and inducing mitochondrial-mediated apoptosis, they also cause T cell death.

  The overall result is a downregulation of the immunological response mediated by cells.[85]

- Azathioprine is metabolised through a variety of enzyme processes. It initially transforms quickly into 6-mercaptopurine.

- Additional 6-mercaptopurine metabolism occurs in three interconnected routes. The enzyme hypoxanthine phosphoribosyl transferase catalyses the formation of the active metabolite, 6- thioguanine.[86]

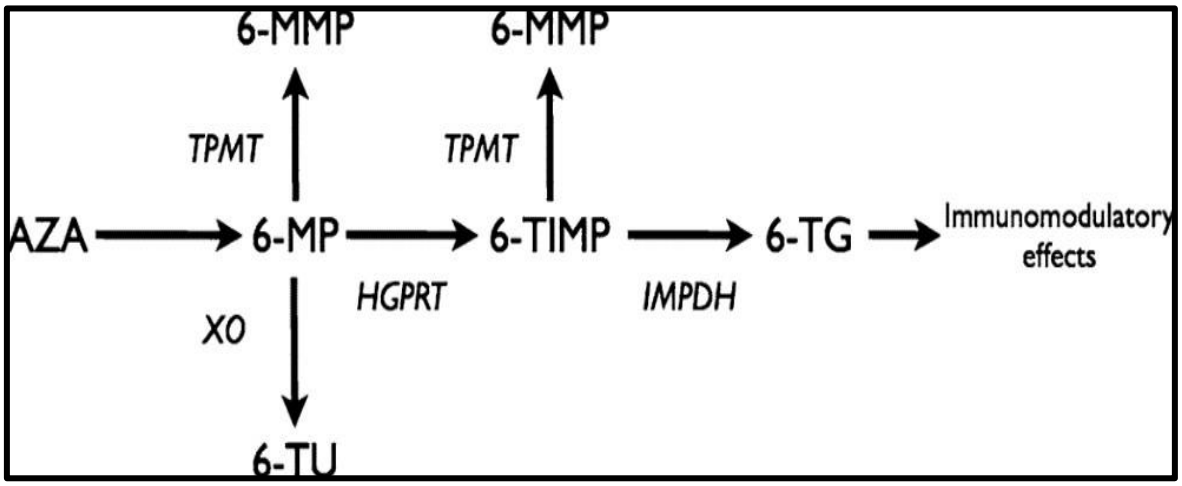


FIGURE: 6 AZATHIOPRINE PATHWAY OF METABOLISM

- 6-MMP 6-methyl mercaptopurine
- 6-TG 6-thioguanine;
- 6-TIMP 6-thioinosine monophosphate;
- 6-TU 6-thioruic acid; HGPRT hypoxanthine guanine phosphoribosyl transferase;
- IMPDH inosine monophosphate dehydrogenase;
- TPMT thiopurine methyl transferase;
- XO xanthine oxidase.[87]

• The evidence shows that AZA/6-MP are efficient treatments for causing and maintaining remission in steroid-dependent illness that does not respond to 5-aminosalicylates. Before beginning AZA medication, it is advised to determine the thiopurine methyl transferase (TPMT) phenotype as serious leukopenia is caused when the medications are administered with lower enzyme levels .[88]

- Most common adverse effects of AZA are:

Gastrointestinal, dermatological and musculoskeletal complications, while serious adverse effects are bone marrow toxicity, pancreatitis, and hepatotoxicity, which leads to the discontinuation of medication. The long-term use of AZA/6-MP is associated with development of lymphomas.[89]

METHOTREXATE

- Methotrexate (MTX) is the most commonly used immunomodulator in rheumatoid arthritis, psoriatic arthritis and psoriasis and are also effective in Crohn's disease and ulcerative colitis.
- MTX is a folate antagonist, which binds to dihydrofolate reductase (DHFR) and inhibits folate synthesis. MTX has several other mechanisms of action that has immunomodulatory effects, including inhibiting IL- 1, IL- 2, IL- 6, and IL- 8; inducing adenosine and also inhibiting purine synthesis.
- The net result of purine metabolism is to reduce cellular and humoral immune response. Cochrane systematic review of the use of MTX in ulcerative colitis has explained that although there appears to be some benefit, it cannot be recommended until further studies are done. MTX is widely used in UC who cannot tolerate AZA or 6-MP but are still requiring steroids and do not tolerate biologic therapies.[112,113]

Adverse effects of MTX include nausea, vomiting, stomatitis and malaise; Serious effects are not common at doses used in ulcerative colitis but may include pulmonary fibrosis, renal failure due to MTX crystal nephropathy, bone marrow toxicity, and hepatotoxicity. It has a risk of teratogenicity, in females of reproductive or child bearing age groups.[92]

CYCLOSPORINE A

- Cyclosporine A (CYA), which is a lipophilic cyclic peptide calcineurin inhibitor, inhibits the production of interleukin-2 and blocks activation of T-lymphocytes by interleukin-2, resulting in downregulation of the cellular immune response.[93]
- Traditionally, it is used for prevention of rejection of solid organ transplant, but it is used off -label for treatment of rheumatoid arthritis and inflammatory bowel disease. In ulcerative colitis, CYA has been found to be effective at delaying the need for colectomy in severe disease that is refractory to steroid therapy, also it has been proved to be a good drug in acute colitis and reduces the demand of steroids but there is uncertainty over its long-term use.

- Due to the high rate of adverse events, CYA is difficult to use in case of inflammatory bowel disease. The most common threatening adverse events attributable to CYA are reversible paraesthesia's, hypo-magnesia, headache, hypertrichosis. More serious adverse events include non-reversible nephrotoxicity, seizure, and serious infections (including pneumocystis carinii pneumonia, community acquired pneumonia, and disseminated viral infections).[94]

TABLE 3: IMMUNOMODULATORS FOR THE TREATMENT OF ULCERATIVE COLITIS.[83]

IMMUNOMODULATORY AGENTS		
DRUGS	THERAPEUTIC USES	CLINICAL PHARMACOLOGY
6-Mercaptopurine	It is employed in the management of mild to severe Crohn's disease and ulcerative colitis, in addition to glucocorticoids and biologics. • Effective in sustaining remission in situ.	• Since it is a Slow-acting drug, it may take months to achieve maximal therapeutic benefit; •Other metabolites have anti-inflammatory properties. • Patients who have IBD, administered with thiopurines have a fourfold greater chance of developing lymphoma.
Azathioprine	• It is used in the treatment of moderate-to severe Crohn's disease and ulcerative colitis, as an adjunct to glucocorticoids and biologics	• It is a prodrug that converts into the active form, 6-mercaptopurine, by nonenzymatic metabolism in the blood.

	• Effective in maintenance of remission	• Patients who have IBD and are administered thiopurines have a fourfold increased risk of developing lymphoma.
Methotrexate	• It is used in the maintenance of remission in Crohn's disease, mainly in steroid-resistant or steroid-dependent disease. • It is used in combination with biologic agents.	Folic acid analogue that has anti-inflammatory activity of unclear mechanism. • It is administered parenterally • Cleared unaltered by the kidney, so inhibition of renal excretion mechanisms may lead to drug toxicity.
Cyclosporine	• Used to treat specific cases of severe Crohn's disease, including fistulizing disease. • It is ineffective in the treatment for the maintenance of remission cases.	• Blood levels need to be monitored since incomplete absorption occurs.
Tacrolimus (FK06)	Used in the treatment of refractory Crohn's disease.	Immunomodulator with higher oral absorption and a mechanism similar to cyclosporine.

6.4. BIOLOGICAL AGENTS

- The development of infliximab for the treatment of Crohn's disease marked the start of the era of biological therapy for inflammatory bowel disease (IBD). Biological therapy once was used only for patients with moderate to severe disease who were not responding to other treatments, but it is now commonly employed as the first-line therapy for moderate to severe disease. Biologicals used in UC include TNF-α inhibitors, integrin receptor antagonists & Janus Kinase (JAK) inhibitors.[95]
- TNF-α inhibitors include Infliximab, Adalimumab, Golimumab and Certolizumab.
- Integrin receptor antagonists include Vedolizumab and Etrolizumab and Tofacitinib is a JAK inhibitor.
- Biological therapies are associated with side effects. TNF-α inhibitor are associated with development of antibodies causing acute reactions and serum sickness, development of lymphomas (non-Hodgkin's and hepato-splenic T cell lymphoma), skin lesions like newly formed psoriasiform eruptions, drug induced lupus syndrome, infections and others like acute liver injury due to reactivation of hepatitis B.
- TNF targeting therapies are contraindicated in congestive heart failure patients. Integrin inhibitors have been associated with cases of progressive multifocal leukoencephalopathy (PML).[95]

TABLE 4: BIOLOGICALS FOR THE TREATRMENT OF ULCERATIVE COLITIS.[83]

Biologics: Anti-TNF-α		
DRUGS	THERAPEUTIC USES	CLINICAL PHARMACOLOGY
Infliximab	In patients who have not responded well to other therapies, it is used in the induction or maintenance of remission in moderate-to-severe Crohn disease or ulcerative colitis.	•It is partly humanized, chimeric anti–TNF-α monoclonal antibody • Usually administered by intravenous infusion. • Patients develop antibodies against the drug
Adalimumab	In patients who have not responded well to other therapies, it is used in the induction or maintenance of remission in moderate-to-severe Crohn disease or ulcerative colitis.	• It is fully humanized anti–TNF-α monoclonal antibody; • Administered subcutaneously • Useful for patients for whom infliximab has lost efficacy or has caused adverse reactions
Certolizumab pegol	In patients who have not responded well to other therapies, it is used in the induction or maintenance of remission in moderate-to-severe Crohn disease or ulcerative colitis.	• It is humanized anti–TNF-α monoclonal antibody bound to PEG to increase plasma t1/2 • It is administered subcutaneously • Useful for patients for whom infliximab has lost efficacy or caused adverse reactions

		•It is a better option in pregnant women due to less drug crossing placental barrier
Vedolizumab	In patients who have not responded well to other therapies, it is used in the induction or maintenance of remission in moderate-to-severe Crohn disease or ulcerative colitis	• Humanized anti-α4β7 monoclonal antibody. • It is given by intravenous infusion. • It causes hypersensitivity reactions.
Ustekinumab	In patients who have not responded well to other therapies, it is used in the induction or maintenance of remission in moderate-to-severe Crohn disease or ulcerative colitis	• Humanized monoclonal antibody against p40 subunit of IL-12 and IL-23 • It is administered subcutaneously. • Long-term safety profile has not yet been established.

6.5.ANTIBIOTICS, PREBIOTICS, PROBIOTICS, NUTRITIONAL THERAPY IN ULCERATIVE COLITIS:

- Antibiotic therapy is commonly used for treating inflammatory bowel disease along the rationale as probiotic and prebiotic therapies.[96]
- Bacterial organisms play an important role in the initiation and perpetuation of inflammatory bowel disease. Meta-analysis of six randomized controlled trials (antibiotic treatment 5–14 days) indicated that patients receiving antibiotics in addition to conventional therapy achieved a higher rate of clinical remission than those receiving placebo and conventional therapy, even the use of antibiotics showed no benefit in maintenance of remission.[97]

- Although omega-3 fatty acid supplementation has been shown to ameliorate chronic inflammatory diseases but no such evidence was found for ulcerative colitis. A meta-analysis of three randomized controlled trials concluded that there was no evidence to support the use of omega-3 fatty acids during periods of remission to prevent relapse for ulcerative colitis.[98]

TABLE 5: ANTIBIOTICS, PREBIOTICS, PROBIOTICS FOR THE TREATMENT OF UC.[83]

Antibiotics		
DRUGS	THERAPEUTIC USES	CLINICAL PHARMACOLOGY
Metronidazole	• Used as adjunctive therapy in mild-to moderate Crohn's disease • Used in conjunction with ciprofloxacin • Used in paediatric IBD	• It has a good therapeutic benefit in Crohn's disease
Ciprofloxacin	• Used as adjunctive therapy in mild-to moderate Crohn's disease • Used in conjunction with ciprofloxacin • Used in paediatric IBD	• It has a good therapeutic benefit in Crohn's disease
Rifaximin	• Used as adjunctive therapy in mild-to moderate Crohn's disease • Used in paediatric Crohn's disease	• It has fewer side effects with this drug compared to metronidazole or ciprofloxacin
Probiotics		
Various types and formulations	• Used in ulcerative colitis and pouchitis, but few clinical trials	The side effects are transient but long-term colonic colonization rarely occurs.

DRUGS USED IN THE PRESENT STUDY

I. PREDNISOLONE

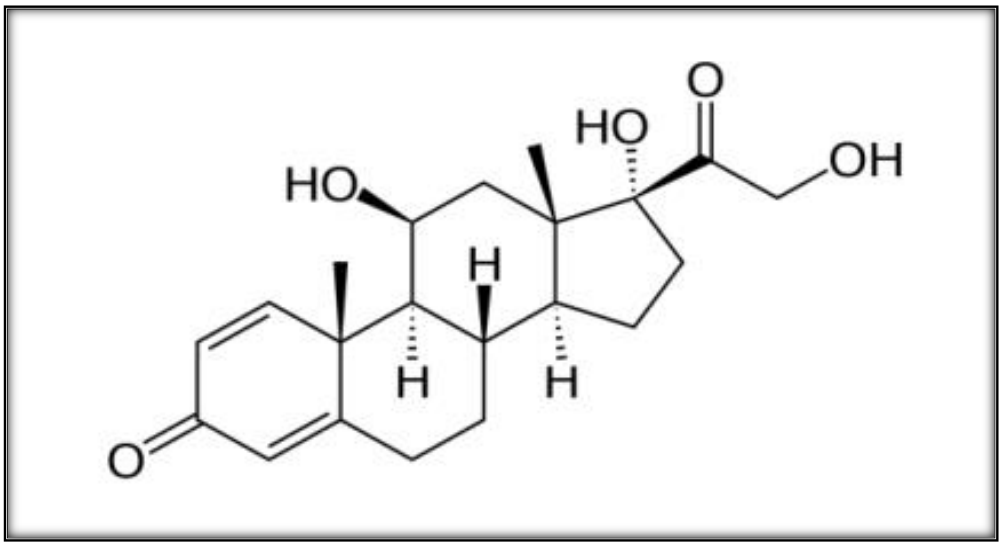


Figure 7: Chemical Structure Of Prednisolone.

- Prednisolone is a glucocorticoid.
- Chemical form:
  11,17-Dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl6,7,8,9,10,11,12,13,14,15,16,17-dodecahydrocyclopenta-phenanthren-3-one.
  Prednisone is a prodrug of prednisolone and is activated by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD)-1.
- The conversion of prednisone into prednisolone occurs rapidly and plasma concentrations of both substances reaches its peaks at approximately 0.5–3 h after prednisone administration.
- Prednisolone has been listed by World Health Organization(WHO) as an essential medicine. It is used to treat a myriad of acute and chronic diseases including asthma, hepatitis, arthritis, systemic lupus erythematosus, and allergic dermatitis. It is also used in the treatment of certain types of allergies, inflammatory conditions, autoimmune disorders and cancers. Compared to hydrocortisone,

prednisolone exhibits significantly higher anti-inflammatory potency with lower sodium retaining ability.[98]

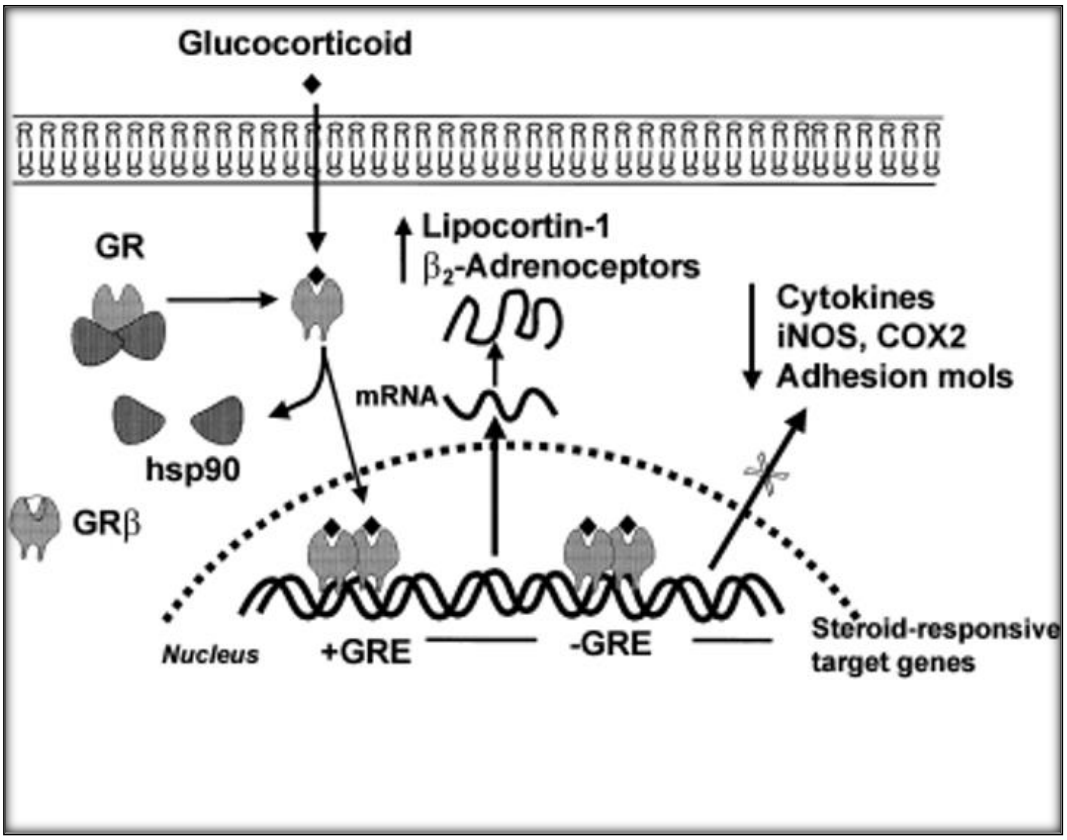


Figure 8: Mechanism of Action of Prednisolone.[100]

MECHANISM OF ACTION

- The mechanism of action of prednisolone is through activation of a cytoplasmic glucocorticoid receptor and subsequent nuclear translocation. In the nucleus, the prednisolone–glucocorticoid receptor complex binds to specific DNA- binding sites known as glucocorticoid response elements (GREs) resulting in gene expression or inhibition. Complex binding to positive GREs leads to synthesis of anti-inflammatory proteins while binding to negative GREs block the transcription of inflammatory genes.
- It decreases the inflammation via suppression of the migration of polymorphonuclear leukocytes and reversing increased capillary permeability. It is metabolized in liver to active metabolite prednisolone, which is then metabolized to inactive glucuronide and sulfated metabolites.[100]

PHARMACOKINETIC PROFILE

- The pharmacokinetics of prednisolone is complex. After oral administration, it exhibits rapid absorption and it has 80%-100% bioavailability. Peak plasma concentrations reaches 1 to 2 hours after oral administration. Distribution of prednisolone is dependent on its protein-binding properties.

- Prednisolone binds reversibly to albumin as well as to a specific α1-glycoprotein named transcortin (corticosteroid binding globulin). Transcortin has a higher affinity but a relatively low capacity for binding prednisolone and thus becomes saturated at therapeutic concentrations because of its relatively lower abundance in plasma. Albumin possesses a lower affinity but a much higher capacity for binding prednisolone and does not become saturated. Ultimately, prednisolone shows higher protein binding (80%-90%) at low concentrations, but lower protein binding (60%-70%) at higher concentrations as transcortin becomes saturated. Similar to many steroid hormones, prednisolone is thought to be metabolized by cytochrome P450 enzyme, specifically CYP3A4.
- The main metabolic pathways are 6-hydroxylation by CYP3A4 along with reduction in 20-keto group of both prednisolone and prednisone. Excretion of prednisolone occurs by urination and approximately 20% of dose gets excreted in its unchanged form.[101]

ADVERSE EFFECTS

Adverse reactions from the use of prednisolone include:

- Increased appetite, weight gain, nausea, and malaise.
- Increased risk of infection.
- Cardiovascular events.
- Hyperglycaemia; patients with diabetes may need increased insulin or diabetic therapies.

- Dermatological effects include facial flushing, bruising/skin discoloration, skin rashes, delayed wound healing, thinning of skin.
- Menstrual abnormalities.
- Electrolytes imbalance : rise in blood pressure, increased sodium and low potassium, leading to alkalosis.
- GI side effects: Inflammation of mucosal lining of colonic epithelium, reversible increase in liver enzymes, and risk of stomach ulcers.
- Muscular and skeletal abnormalities, such as muscle weakness/muscle wasting, long bone fractures, tendon rupture etc.
- Neurological effects, includes convulsions, vertigo , behavioural disturbances
- Nasal septum perforation and bowel perforation.

Withdrawal from prednisolone after long-term or high-dose use can lead to adrenal insufficiency.[102]

USES

- Prednisolone is a corticosteroid with predominant glucocorticoid and low mineralocorticoid activity, It is used in the treatment of inflammatory and autoimmune conditions such as asthma, uveitis, pyoderma gangrenosum, rheumatoid arthritis, urticaria, angioedema, ulcerative colitis, pericarditis, temporal arteritis and Crohn's disease.
- It is also used in the treatment of Bell's palsy, multiple sclerosis, cluster headaches, vasculitis, acute lymphoblastic leukaemia, autoimmune hepatitis, systemic lupus erythematosus, Kawasaki disease, dermatomyositis, and sarcoidosis.

Prednisolone in lower doses is used in cases of primary adrenal insufficiency (Addison's disease).

- Corticosteroids inhibit the inflammatory response to a variety of agents and it is assumed to delay or slow the wound healing. They inhibit the oedema, fibrin deposition, capillary dilation, leukocyte migration, capillary proliferation, fibroblast proliferation, deposition of collagen, and scar formation with inflammation.[103]

PREDNISOLNE IN RELEVANCE TO PRESENT STUDY:

In view of the above-mentioned information, prednisolone is considered a safe drug and has major anti-inflammatory activity. One of the animal study shows that inhibition of GRE (Glucocorticoid Response Elements) down-regulates pro inflammatory mediators associated with chronic colitis and decreased intestinal impermeability. Moreover, there is a paucity of studies looking for the direct effect of prednisolone in ulcerative colitis. Therefore, this study is planned with the intension to determine the effect of prednisolone in experimental colitis.

DRUG USED IN THE PRESENT STUDY

II. *Mucuna pruriens:*

- *Mucuna pruriens* (Fabaceae family) is commonly known as cowage plant / kapikacho and is a popular Ayurvedic and Unani medicine.[6] *Mucuna pruriens* seed extract has been used to treat Rheumatoid arthritis owing to its anti-inflammatory property.[8]

PHARMACOLOGICAL ACTIONS

Antivenom Activity

Mucuna pruriens seed was found to have reduction in cardiovascular and neuromuscular depressant effect of Naja sputatrix venom on rat possessing antivenomic activity.

Hypoglycaemic Activity

Hypoglycaemic activity of Mucuna pruriens suggested that there was a significant reduction in glucose load from 127 mg/dl to 75 mg/dl along with reduction in creatinine and cholesterol levels.

Aphrodisiac Activity

Mucuna pruriens, in the formulation of Kapikacho Churna is found to be most effective in decreased sperm count. It shows good results in seminal parameter such as semen motility, sperms volume and pH of semen. It also showed significant improvement in nonprogressive sperm (NPS).

Antioxidant Activity

Alcoholic extract of Mucuna pruriens seeds showed excellent antioxidant activity when compared with total phenol content and ascorbate. Several parts of Mucuna pruriens plant contain phenols which have antioxidant property.

Antimicrobial Activity

Methanolic extract of Mucuna pruriens found to have significant result against Gram+ve and Gram-ve organism. This extract is extremely active against Salmonella typhi, Shigella dysenteriae, Escherichia coli and Bacillus subtilis.

Anti- Parkinson's Activity

Mucuna pruriens seed extract contains significant level of levodopa, which is a direct precursor of the neurotransmitter dopamine. It is used in the treatment of Parkinson's disease. Extract of Mucuna pruriens seeds with n-propanol shows effective response in neuroprotective test which enhance the growth of dopamine neurons in brain culture. Therefore, it is considered as superior to pure form of L-DOPA in the treatment of Parkinsonism.

Pharmacological actions such as Anti-venomic property, Hypoglycaemic activity, Aphrodisiac activity , Anti-oxidant activity, Anti-microbial activity, Anti-parkinson's activity are summarized above.[104]

- Drugs used in treatment of ulcerative colitis are corticosteroids, TNF- α antagonists, anti-integrin agents and sphingosine-1-phosphate receptor agonists. These drugs are unable to arrest the pathology of inflammatory bowel disease or treat them to full remission.[105]
- Also, their adverse effects profile possess danger to the patient receiving these drugs for life.
- Hence, there is an unmet need for more scientific research in developing efficacious and safe drugs in the fight against inflammatory bowel disease particularly in the treatment of ulcerative colitis.[10] Therefore, this study was conducted to find how effective is *Mucuna pruriens* for Inflammatory bowel disease particularly ulcerative colitis.

EXPERIMENTAL MODELS OF COLITIS

- Experimental models play an important tool for testing of new drugs and to study the pathogenesis in IBD. The most important characteristics of a successful model should include features viz. gut should exhibit morphological alterations, inflammation, signs and symptoms, pathophysiology and course identical to the human IBD.

  Experimental models are broadly classified as follows:

- Chemically induced experimental models
- Spontaneous colitis models
- Gene knockout (KO) models
- Transgenic mouse and rat models
- Adoptive transfer models
- Less frequent microbial models.[106]

EXPERIMENTAL MODEL USED IN THE CURRENT STUDY:

2,4,6-Trinitrobenzene Sulfonic Acid

- The first developed model in rats was TNBS. In this model, ethanol is required to break the mucosal barrier, whereas TNBS haptenizes colonic autologous or microbiota proteins, making them immunogenic in nature.
- CD4+ T cells have been shown to play an important role in chronic TNBS colitis and hence this model is useful to study T-helper-cell-dependent mucosal immune responses.
- The most important characteristic of the TNBS model is that colonic inflammation is permanent, because the immune cells are activated by TNBS.
- The mucosal antigens are continued to be stimulated even after the trinitrophenyl (TNP)-haptenized proteins have disappeared.
- Because of the involvement of IL-12 driven Th1- mediated inflammation, the chemical model of inflammation mimics like Crohn's disease.

- Trinitrobenzene sulfonic acid (TNBS) is an hapten, binds to tissue protein and converts into an antigen and then induces a number of immunologic responses. DSS induced colitis is the result of a disruption in epithelial barrier, whereas TNBS induced colitis is a delayed-type hypersensitivity reaction to haptenized proteins.
- Leukotriene B4 (LTB4) and the monohydroxy fatty acids 5-HETE, 12-HETE and 15-HETE are major inflammatory mediators involved in this TNBS-induced colitis.
- For the administration of the TNBS, Ethanol is used in high concentration as a vehicle.
- The TNBS can enter the mucosa to induce colitis where ethanol acts as a mucosal barrier breaker and it binds to the amino group of lysine and it will modify the cell surface proteins. [107]

CHEMICALLY INDUCED EXPERIMENTAL MODEL OF COLITIS:

1. **DINITROBENZENE SULFONIC ACID**

- Dinitrobenzene sulfonic acid (DNBS) is a hapten, which induces the features of colitis and colonic inflammation. It involves additional active nitro group that binds more readily at the level of lower concentrations. It also binds to the e-amino group of lysine and is more selective hence used in the induction of colonic inflammation.

- The clinical presentation of colitis are presented in this model are bloody diarrhoea and significant reduction in the body weight. Administration of DNBS (intrarectally) results in reduced food intake, increased weight of the colon and altered stool consistency. DNBS causes overproduction of nitric oxide (NO) due to induction of inducible nitric oxide synthase (iNOS). This model is characterised by transmural necrosis with extensive morphological disorientation, immune cell infiltration and oedema in the submucosa of the colon. [108]

2. **DEXTRAN SULFATE SODIUM**

- Dextran sulfate sodium (DSS) is a synthetic sulfated polysaccharide consists of dextran and sulfated anhydro glucose unit. It has highly water solubility. The colitogenic potential of DSS mainly relies on its molecular weight. In general, the molecular weight of 36–50 kDa is used for inducing colitis.

- The low molecular weight DSS (5 kDa) results in milder colitis, while the high molecular weight of DSS (500 kDa) does not results into colonic injury. DSS has high negative charge due to the presence of sulfate group. It is toxic to the epithelium of colon and triggers mucosal erosions that ultimately leads to the disruption of barrier integrity.
- The anticoagulant property exacerbates the bleeding of intestine. It has been suggested that DSS forms nanolipocomplexes with medium-chain-length fatty acids in the colon.[109]

3.RECURRENT TNBS-INDUCED COLITIS

- In this model, colitis is induced by repeated administration of TNBS intrarectally resulting in the development of chronic intestinal inflammation. Increase in weight and thickness is evident in the distal part of the inflamed colon. In histopathological examination, it shows increased inflammatory cellular infiltration, which consists of $CD4_+$ and $CD8_+$ T cells, macrophages, granulocytes and mast cells, irregular crypts, and loss of Goblet cells.[110]

4. OXAZOLONE- INDUCED COLITIS

- Oxazolone and ethanol when administered intrarectally induces Th2-mediated acute colitis. This model resembles ulcerative colitis and is limited to the distal part of the colon, like TNBS-induced colitis model. Oxazolone-induced colitis has been recommended to be dependent on the presence of IL-13 producing invariant NK-T cells.[111]

5. ACETIC ACID INDUCED COLITIS

- Acute inflammation is induced by acetic acid which is limited to the colon and produces certain clinical features of UC. The colonic injury relies on the length and concentrations of exposure of acetic acid and is related to the oedema and necrosis in epithelial cells.
- In this model, mucosa and submucosal inflammation is linked with activation of NF-kB and inflammatory mediators. Acute injury in this model is caused by transient local ischemia.[112]

6. CARRAGEENAN INDUCED COLITIS

- The proinflammatory agent Carrageenan, with high molecular weight sulfated polygalactan, evolved from numerous species of red seaweeds (Rhodophyceae), including *Chondrus*, *Eucheuma* and *Gigartina*. Carrageenan activates innate immune pathways of inflammation that mimics ulcerative colitis in which BCL10 and TLR-4 plays a crucial role.[113]

7. INDOMETHACIN-INDUCED ENTEROCOLITIS

- In rodents, Indomethacin causes ulceration in small intestine and colon in a dose-dependent manner. The chronic ulceration is present in the mid portion of the small intestine rather than the ileum.
- Although the transmural inflammation and small bowel ulceration have some similarity to Crohn's disease, it involves small and large intestines and is associated with extraintestinal lesions.[114]

8. IODOACETAMIDE

- Iodoacetamide is a blocker of enzymes that comprise of SH-group (sulfhydryl) in the colonic area. GSH contains SH-group and plays an important role in the protection of gastric mucosa.
- Blockade of GSH by the iodoacetamide produces mucosal injury by decreasing the amount of SH compounds and induces ulcerative colitis. [115]

SPONTANEOUS COLITIS MODELS

1. C3H/HejBir mice

C3H/HejBir is a derivative of selective breeding of C3H/Hej mice with colitis known to develop perianal ulcers and colitis. Colitis is confined to ileocecal lesions and right side of the colon. It occurs spontaneously in the 3rd to 4th week and then disappears after 10 to 12 weeks. Ulcers and crypt abscesses are observed, but thickening of the intestinal wall and granulomas are not visible. Increased levels of interferon gamma IL-2 and (IFNγ) have been found in the lamina propria of lymphocytes, suggesting Th type-1 response. This model when used with inducible colitis model, has proven valuable results for identifying and studying the genetic susceptibility factors.[116]

2. SAMP/Yit mice

SAMP/Yit mice are a sub-strain derived from the selective breeding of AKR mice. Colitis is induced in all mice by the 30th week, with skip lesions, and crypt abscesses. Laminar propria of the lymphocytes when stimulated will produce higher levels of IFN-α and TNF-γ than those of AKR mice. Antibody blockade of the vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, induce inflammation in the SAMP/Yit.[117]

GENE KNOCKOUT (KO) MODELS

1.Interleukin-2 KO/IL-2 receptor (R)α KO mice

In mice with a distorted IL-2 gene, the small intestine remains intact, whereas the colon (from rectum to caecum) is adversely affected with cell wall thickening and ulcers. In histopathological examination, mucin depletion, crypt abscesses and dysplasia of the epithelial cells as well as infiltration of activated B cells and T cells are noted. IL-2-dependent T cells are generated in the thymus at an early stage after birth, a deficit of these regulatory T cells induces a Th type-1 response in this model.[118]

2. T cell receptor (TCR) α mutant mice

Most animal models have Th type-1 immune responses (predominantly IFN-α and TNF-γ). This model is suggested to have a Th type-2 immune response (predominantly IL-4, IL-5) due to reduction in the binding affinity between the major histocompatibility complex (MHC) and TCR, due to the secretion of IL-4 and depletion of the α chain.

Change in the stool consistency, persistent inflammation and hypertrophy of the colon are seen in 16 weeks after birth, but the small intestine remains intact. Decrease in the number of goblet cells and crypt abscesses, infiltration of lymphocytes, plasma cells, and neutrophils and hyperplasia of the colonic epithelium, are also observed. Auto-antibodies are produced because of the immunological disorder and assumed to work against, rather than promoting the inflammation. It is suggested that only in TCRα -/- mice the presence of colitis is permanent.[118]

3. IL-10 KO mice models

IL-10 produced by B cells, T cells, macrophages, thymic cells, and keratinocytes, down regulates the function of macrophages, NK cells and T helper (Th)-1 cells. Inflammation occurs in the entire part of the intestine of IL-10 -/- mice. In the colon, degeneration of the epithelium, infiltration of IgA- producing plasma cells, goblet cell depletion and an increase in MHC class II expression are observed.[119]

Similar to IL-2 -/- mice model, there is an activation of CD4 + Th1 cells and the depletion of the regulatory T cells, which are presumed to be the cause of the inflammation. Colitis that developed in the IL-10 -/- mice emerged into two distinct phases: IL-12 played an important role in early colitis, whereas its synthesis and the absence of IL-4 and IL-13 in late disease indicated that the immune mechanisms sustained chronic inflammation. Therefore, it is suggested that there is an impairment in the regulation of macrophages, but not the T cells.[119]

4. TNF-3' Untranslated Region (UTR) KO Mice

In mice, colitis would occur with over expression of human TNFα. There is an adenine/uracil-rich element (ARE) consisting of AUUUA repeats in the 3V-UTR area of IL-2, and granulocyte macrophage–colony-stimulating factor (GM–CSF) which destabilizes the mRNA of the cytokines. Mice deficient in the adenine/uracil-rich element (ARE) of TNFα shows high levels of serum TNFα and have colitis.[120]

5. Trefoil Factor-Deficient Mice

Intestinal trefoil factors (ITFs) are peptides produced by mucus cells of the intestine after inflammatory damage. Mice with disrupted ITF shows decreased epithelial regeneration and severely impaired mucosal healing.[121]

TRANSGENIC MOUSE & RAT MODELS

1. IL-7 Transgenic Mice

It has been demonstrated that IL-7 within the serum of ulcerative colitis patients influences the proliferation and differentiation of T cells in the thymus. Further investigation of IL-7 transgenic mice, revealed that acute colitis occurred in 1 to 3 weeks of age along with infiltration of T cells, CD4 + T cells and neutrophils in the intestine followed by proctoptosis with anal bleeding at 8–12 weeks of age. This is therefore, a chronic colitis model that mimics the human ulcerative colitis. It is suggested that, in the acute phase, the excessive secretion of IL-7 induces activation of the mucosal lymphocytes to induce colitis. While in the chronic phase, apoptosis of the activated lymphocytes results from the lack of IL-7 presumed to be the cause of colitis.[122]

2. (STAT)-4 Transgenic Mice

Of the seven Signal Transduction and Activator of Transcription (STAT) family, each works for several cytokines. STAT-4 is particular to the signal transduction of STAT-4 -/- mice and IL- 12 mice which are similar to KO model of Th type-1 colitis.[123]

3.HLA-B27 Transgenic Rats

It is a model of transgenic rat for human HLA-B27 and β2-micro-globulin which forms spontaneous IBD that affects the stomach, ileum, and the entire colon. Mostly mucosal infiltration and crypt hyperplasia characterizes the IBD. This model has been used widely to demonstrate that various bacterial species can induce various types of pathology in gastritis and colitis. Increased levels of IL-2 and IFNγ in the lamina propria of lymphocytes, indicates a Th type-1 response.[124]

ADOPTIVE TRANSFER MODELS

1. Heat Shock Protein (hsp) Transfer Colitis (60-Specific CD8 T Cells)

Colitis in these mice required the presentation of hsp60 on MHC class I and depend on a functional role of TNFα. Initial demonstration of this model indicates that an autoimmune hsp60 CD8 + T cells reactive to cellular hsp60, mediates the pathogenesis of colitis.[125]

2. CD45RB Transfer Model

The adoptive transfer of CD4 + T cells expressing high levels of the surface molecule CD45RB (CD4 +CD45RBhi) into severe combined immune deficient (SCID) recipients (i.e., CD4 + CD45RBhi T cells) will result in muscle wasting and chronic non-bloody

diarrhoea. The disease is progressive and deadly. The histopathologic changes are like other models of colitis, and is confined to the colon, which is significantly thickened due to hyperplasia of the colon.[126]

LESS FREQUENTLY USED MODELS

1. **Peptidoglycan–Polysaccharide (PG-PS) Colitis**

   The intramural injection of bacterial cell wall component PG-PS into the colon of rats induces enterocolitis with infiltration of macrophages and neutrophils along with thickening of the colon. PG–PS increases mucosal permeability and MPO activity. It also enhances collagen synthesis and nitric-oxide production. This model shows that the cell wall components of non-pathogenic resident enteric bacteria are adequate to induce colitis.[127]

2. **Radiation Induced Colitis**

   Radiation Induced Colitis is a relatively novel model. It is a combination of gamma irradiation and MHC class II deficiency in mice resulting in effective infiltration of colitis.[128]

3. **Germ-Free Mice Colitis**

   The germ-free mice model demonstrates characteristics similar to human ulcerative colitis when microbial isolates from the faeces of genetically identical mice are introduced. Colitis occurs in the germ-rich colon under specific pathogen-free conditions, but not under germ-free conditions.[129]

MATERIALS & METHODS

Source of Data

MATERIALS

Mucuna pruriens seed extract was obtained from Natural Remedies Pvt, Limited, Bengaluru.

Tri Nitro Benzene Sulfonic Acid (TNBS) solution was procured from a standard reagent supplier. (Rajendra Traders Pvt. Ltd, Bangalore)

Prednisolone tablets were procured from the pharmacy connected to the teaching hospital of the institution.

Based on the human dose using the conversion table of Pagets and Barnes,

Dose of prednisolone was calculated.[19]

Dose of *Mucuna pruriens* was calculated.

METHODOLOGY

Adult male Wistar rats weighing 200 ± 20g were recruited from the Central Animal House of the institution. Prior to the experimentation day, they were acclimated to a 12-hour cycle of light and darkness over the course of seven days. All rats were kept on standard rat food pellets and free access to water. The Institutional Animal Ethics Committee, which was constituted in accordance with the rules and regulations of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) New Delhi, approved the study.

The present study was an experimental study in which male Wistar rats were administered TNBS to induce Colitis.

Study Design:

Rats were divided randomly into 4 groups, with (n=8) eight rats in each group. Different groups with drug dosages are provided in Table 6:

Group	Treatment	Route of Drug Administration	Dose [for 14 days]
I	Naïve group (without Colitis): distilled water	Per Oral	0.5ml
II	Control group (with induction of Colitis): distilled water	Per Oral	0.5ml
III	Standard group: Prednisolone	Per Oral	4.5 mg/ kg. [19]
IV	*Mucuna pruriens* group	Per Oral	400mg/ kg [18]

EXCLUSION CRITERIA:

Animals which do not show the signs of colitis (weight loss, per rectal bleeding) on day 1 after TNBS administration were excluded from the study.

INDUCTION OF COLITIS:

Rats were randomly allocated to each group. Experimental approach was followed by the technique demonstrated by Morris et al.[20] To confirm the colitis induction technique and gain expertise with the operation, a pilot trial was
first performed.

On day of induction, anaesthesia with thiopentone sodium in a dose of 30 mg/kg was administered intra peritoneally (IP) before the procedure.[21] A 6 French infant feeding tube was used to administer TNBS at a dose of 50 mg/kg diluted in 0.25 ml, of 50% Ethanol (v/v) 8 cm proximal to the anus. For two to three minutes, the Trendelenburg position was maintained. Following induction, experimental animals were transferred in cages with free access to food and water.

Naive group received distilled water enema in a dose of 0.25ml. Treatment was started 2 hours after induction of colitis and was administered every 24 hours for a duration of 14 days. Throughout the experimental period, evaluation was carried out by assigning scores for various parameters. Daily body weight measurements from 0 to 14 days were used to determine the percentage of weight loss. Stool consistency was monitored every day for 14 days. Stools were observed for presence of blood through visual inspection of faecal matter and peri-anal area 24 hours after induction as well as on days 7 and 14.

According to the change in body weight and clinical indicators such faecal blood and stool consistency, the disease activity index (DAI) was measured. On day 14, the rats were sacrificed and colonic tissues were isolated for histopathological examination.

Blood Collection:

3ml of blood was collected by cardiac puncture (lateral approach technique). Animals were anaesthetised with thiopentone sodium in a dose of 30 mg/kg administered intra peritoneally before the procedure.

Euthanasia of Rats

After the experimental period (day 15), all animals were euthanised using overdose of thiopentone sodium by intraperitoneal route (120 mg/kg, 3- 5 times anaesthetic dosage (as per the rules and regulations of CPCSEA).[21]

Collection of tissue sample:

Animals were euthanised as per the procedure mentioned above. Colon sample was collected by dissecting out the descending colon. The descending colon was identified as a part of the colon which was distal to the splenic flexure and around 8 cm proximal to the anus. The specimen was dissected out and washed in cold saline thoroughly. 4 cm of the colon sample was fixed in 10% formalin and sent for histopathological scoring.

EVALUATION:

During experimental period:

Percentage of change in body weight and Disease Activity Index (DAI) [22,23]

Analysis was done on the body weight as well as the % change in body weight. A sensitive indicator for assessing the severity of colitis is DAI.

DAI was evaluated primarily on the changes in body weight and clinical signs including faecal blood and stool consistency.

TABLE 7: Combined scores of % Weight loss, Stool consistency and bleeding.

SCORE	WEIGHT LOSS %	STOOL CONSISTENCY	OCCULT/GROSS BLEEDING
0	(-)	Well-formed pellets	Absent
1	1-5%	Well-formed pellets	Absent
2	5-10%	Loose pasty stool which do not stick to anus	Streaks of blood
3	10-20%		
4	>20%	Watery stools- liquid stools that stick to anus	Gross bleeding

The DAI value, which ranges from 1 (Healthy) to 4, was calculated by adding the three scores and dividing them by three (maximum colitis activity). The average DAI was calculated by using scores given for each parameter (DAI scores were calculated on days 1,7 and 14).

Food Intake

Total food intake per day per group was calculated by measuring the weight of the standard rat chow pellets before placing the pellets in the cage for feeding and 24 hours after placement. This was done for 14 days.

EVALUATION AFTER EXPERIMENTAL PERIOD

Histopathology study

A section of the inflamed colon was embedded in paraffin after being fixed in 10% phosphate-buffer formaldehyde. The tissue was cut into 5 m slices using a microtome. Haematoxylin and eosin staining was used to examine the sections by light microscopy.

Microscopic examination of the colonic sections was carried out by a pathologist who was blinded to the treatment regimen.

Sections were examined for hallmarks of inflammation, including neutrophil infiltration and preservation of cryptic architecture.

TABLE 8: Histopathological disease score.[23]

Grade 0	Normal colonic mucosa
Grade 1	Loss of one-thirds of the crypts
Grade 2	Loss of two-thirds of the crypts
Grade 3	The lamina propria is covered with a single layer of epithelium and mild inflammatory cell infiltration is present
Grade 4	Erosions and marked inflammatory cell infiltration are present

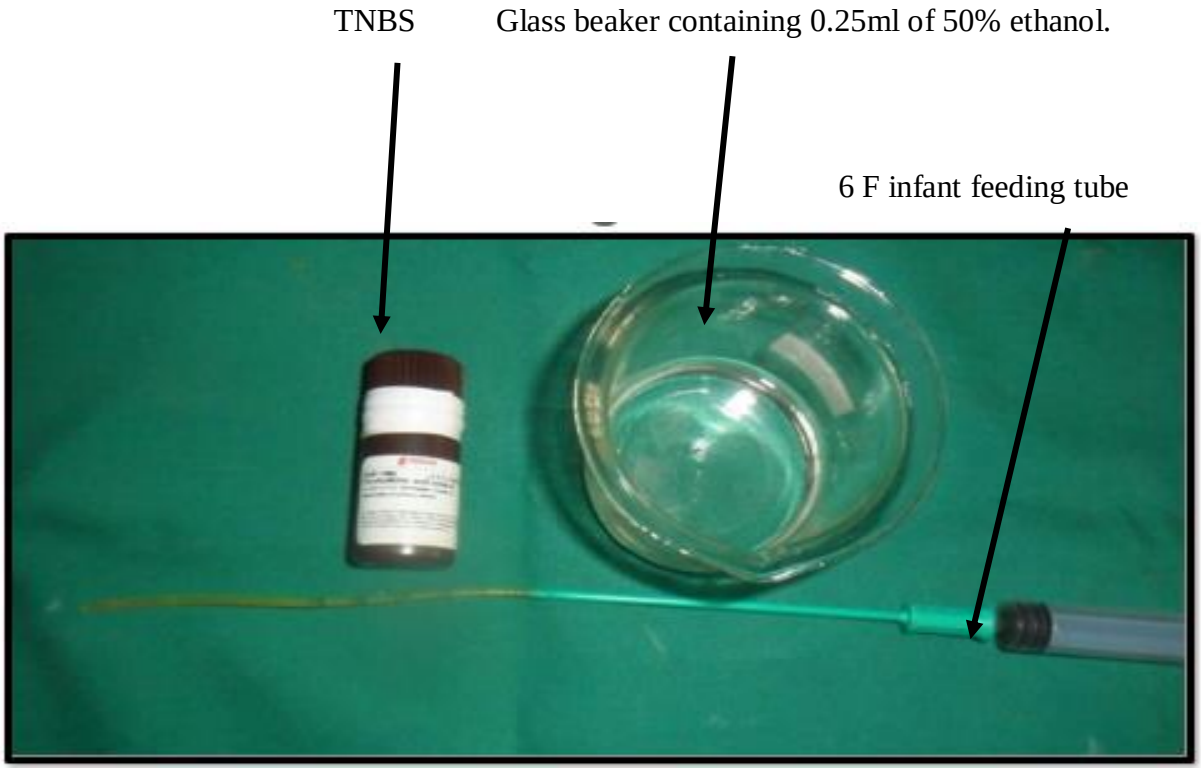


Fig no. 9: Materials required for the induction of colitis

Fig. No 10: Rats After the Induction of Colitis with TNBS.

Statistical analysis

The experimental results are expressed in Mean ± SEM. One-way ANOVA, used to evaluate the significant difference between the treatment group and the colitis-control group for change in body weight, followed by post hoc Dunnett's and Bonferroni's test. Graph Pad Prism 9.0 software was used with a statistically significant p value of 0.05

RESULTS

Data generated in the study were compiled into an excel sheet and were analysed using appropriate statistical tests.

The study results were depicted under the titles of physical parameters, biochemical parameters and histopathological examination by using graphs and tables for easy understanding.

I. Physical Parameters

1. Change in body weight

Throughout the course of the study, daily body weight measurements of the rats were taken. An important component that corresponds with the development of colitis is body weight.

Mean Body Weights of all the groups were comparable at baseline. On day1, the mean body weights (in grams) of normal control, Colitis control, prednisolone group and *Mucuna pruriens* group were 214.875±2.39, 227.375±3.40, 231.375±4.12, 220.00±3.81 respectively. One-way ANOVA revealed that there was no significant difference between various groups at baseline.

On 5^{th} day, the mean body weights (in grams) of normal control group, Colitis control, prednisolone group and *Mucuna pruriens* were 220.625±2.23, 216.25±3.42, 229.375±4.22, 213.75±3.97 respectively. A One-way ANOVA followed by post-hoc Dunnett's test showed that the body weight in Colitis control group was reduced significantly when compared to normal control group ($p<0.05$).

On day 9, the mean body weights (in grams) of normal control group, Colitis control, prednisolone group and *Mucuna pruriens* were 227.50±2.24, 208.875±2.99, 232.75±3.92, 213.875±4.35 respectively. A One-way ANOVA followed by post-hoc Dunnett's test showed that the body weight in Colitis control group was significantly lower than normal control group ($p<0.05$)

On day 11, the mean body weights (in grams) of normal control group, Colitis control, prednisolone group and *Mucuna pruriens* were 231.875±2.37, 204.375±2.97, 234.75±3.72, 215.125±3.97 respectively. A One-way ANOVA followed by post-hoc

Dunnett's test showed that the body weight in prednisolone group and *Mucuna pruriens* group were significantly higher compared to Colitis control group ($p<0.05$).

At the end of the study (day 14), the mean body weights (in grams) of normal control group, Colitis control, prednisolone group and *Mucuna pruriens* were 237.50±2.62, 200.25±2.89, 237.625±3.82, 217.50±3.60 respectively. A One-way ANOVA followed by post-hoc Dunnett's test showed that the body weight in prednisolone group and *Mucuna pruriens* group improved significantly when compared to Colitis control group ($p<0.05$). (Table no. 9, Graph 1

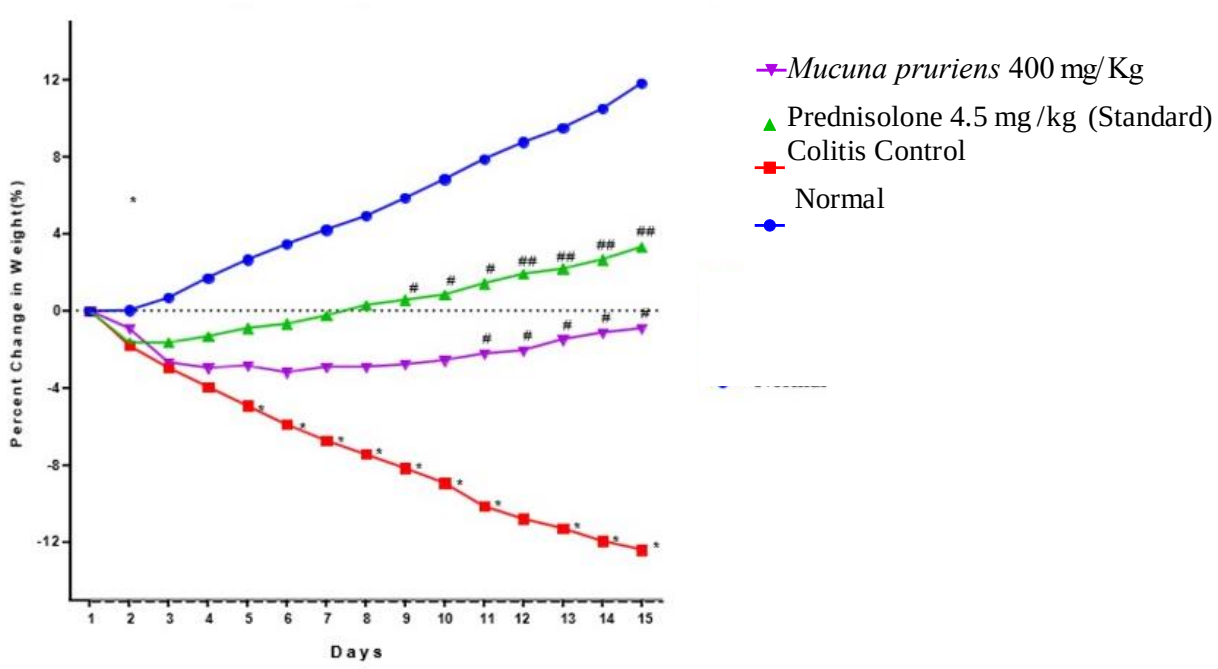
Table 9–: Effect of test drugs on body weight of the animals, with percentage change in weight.

	NORMAL CONTROL		**COLITIS CONTROL**		**STANDARD DRUG – PREDNISOLONE 4.5mg/kg**		**TEST DRUG- *Mucuna pruriens.***	
DAYS	Wt. in gm (Mean ±SEM)	% Change	Wt. in gm (Mean ±SEM)	% Change	Wt. in gm (Mean ±SEM)	% Change	Wt. in gm (Mean ±SEM)	% Change
1	214.875±2.39	0.0	227.375±3.40	0.0	231.375±4.12	0.0	220.00±3.81	0.0
2	215.00±2.19	0.1	223.375±3.28	-1.8	227.625±4.14	-1.6	218.00±3.83	-0.9
3	216.375±2.37	0.7	220.75±3.37	-2.9	227.625±4.11	-1.6	214.125±3.76	-2.7
4	218.625±2.31	1.7	218.5±3.50	-3.9	228.375±4.16	-1.3	213.50±3.63	-3.0
5	220.625±2.23	2.7	216.25±3.42	**-4.9***	229.375±4.22	-0.9	213.75±3.97	-2.8
6	222.375±2.44	3.5	214.0±3.44	**-5.9***	229.875±4.29	-0.6	213.00±3.94	-3.2
7	224.00±2.57	4.2	212.125±3.32	**-6.7***	230.875±4.16	-0.2	213.625±4.32	-2.9
8	225.50±2.43	4.9	210.5±3.18	**-7.4***	232.125±3.89	0.3	213.625±4.37	-2.9
9	227.50±2.24	5.9	208.875±2.99	**-8.1***	232.75±3.92	**0.6#**	213.875±4.35	-2.8
10	229.625±2.37	6.9	207.125±3.15	**-8.9***	233.375±3.72	**0.9#**	214.375±4.22	-2.6
11	231.875±2.37	7.9	204.375±2.97	**-10.1***	234.75±3.72	**1.5#**	215.125±3.97	**-2.2#**
12	233.75±2.51	8.8	202.875±2.69	**-10.8***	235.875±3.79	**1.9##**	215.50±3.92	**-2.0#**
13	235.375±2.58	9.5	201.75±2.7	**-11.3***	236.50±3.84	**2.2##**	216.75±3.92	**-1.5#**
14	237.50±2.62	10.5	200.25±2.89	**-11.9***	237.625±3.82	**2.7##**	217.50±3.60	**-1.1#**

Values are expressed as Mean± SEM, n=8. One-way ANOVA followed by post hoc Dunnet's test and Bonferroni's test.

***p<0.05 in comparison with normal group. #p<0.05, ##p<0.005 in comparison to colitis control group.**

Graph 1: Percentage change in body weight among various groups



Values are expressed as Mean± SEM, n=8. One-way ANOVA followed by post hoc Dunnet's test and Bonferroni's test.

***p<0.05 in comparison with normal group. #p<0.05, ##p<0.005 in comparison to colitis control group.**

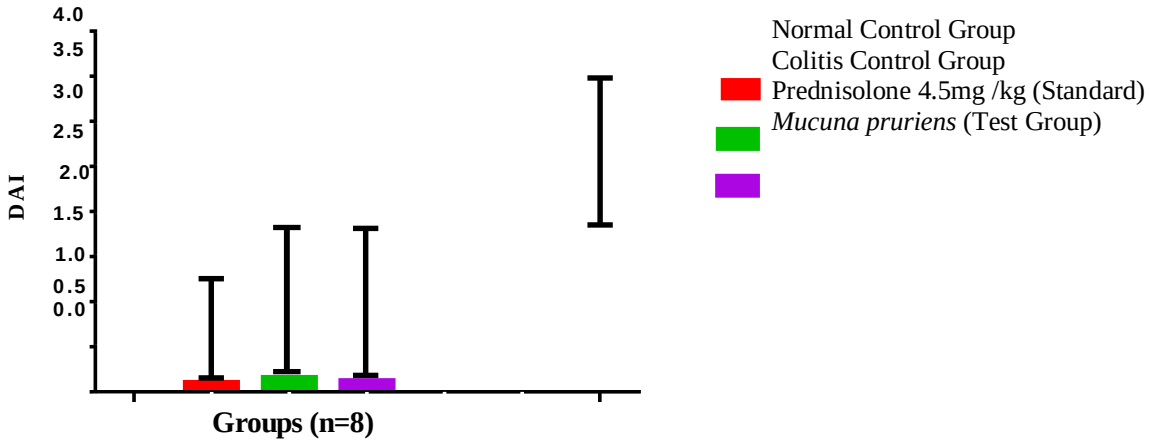
DISEASE ACTIVITY INDEX

Compared to the normal group the scores of DAI in colitis-control group was significantly high depicting that colitis-control group showed significant body weight loss along with blood in the stools and diarrhoea.

On days 1, 7, and 14, the DAI values were recorded. On Day 1 values did not find to differ significantly among the groups. On day 7, only prednisolone found to have significant improvement in DAI scores when compared to Colitis control group. On day

14, both the treatment groups (prednisolone and *Mucuna pruriens*) showed significant improvement in DAI scores when compared to Colitis-control group.

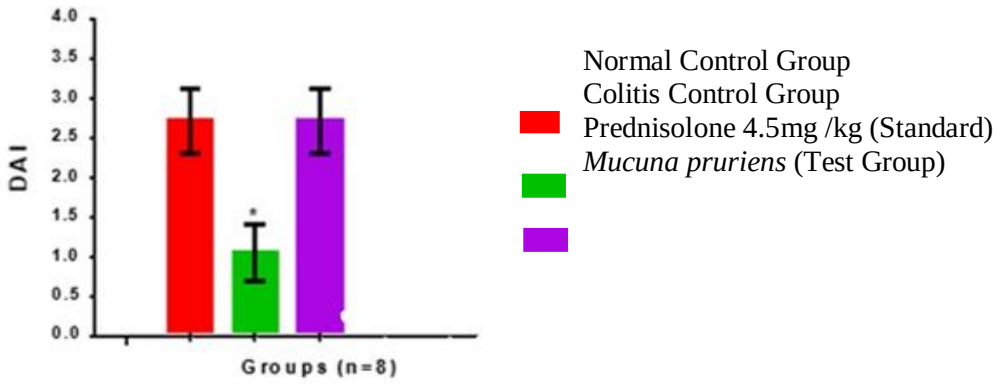
Graph 2: DISEASE ACTIVITY INDEX DAY 1



Disease Activity Index (DAI) as recorded on day 1 of experiment.

Values are expressed as Mean± SEM, n=8. One-way ANOVA followed by post hoc Dunnett's test and Bonferroni's test.

Graph 3: DISEASE ACTIVITY INDEX DAY 7

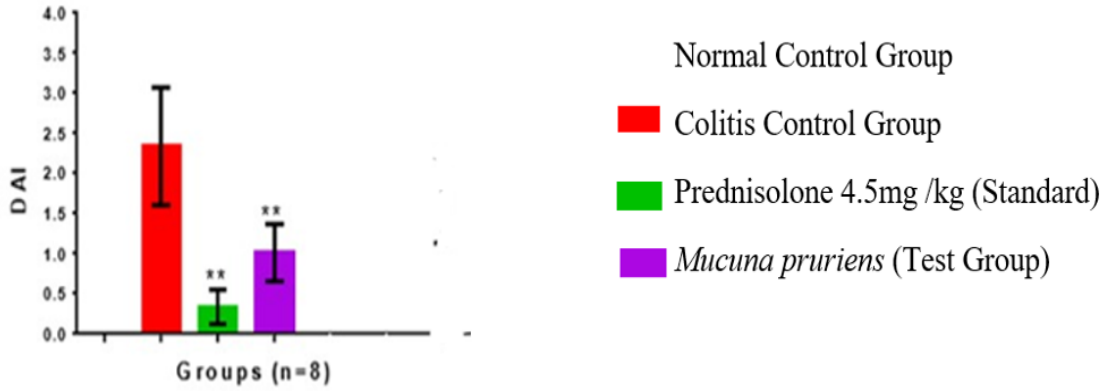


Disease Activity Index as recorded on 7th day of experiment

Values are expressed as Mean ±SEM; n=8. One-way ANOVA followed by pot-hoc Dunnett's test and Bonferroni's test.

***p<0.05 in Comparison with the Normal group; # p<0.05, ## p<0.005 in Comparison to Colitis-control group.**

Graph 4: DISEASE ACTIVITY INDEX DAY 14

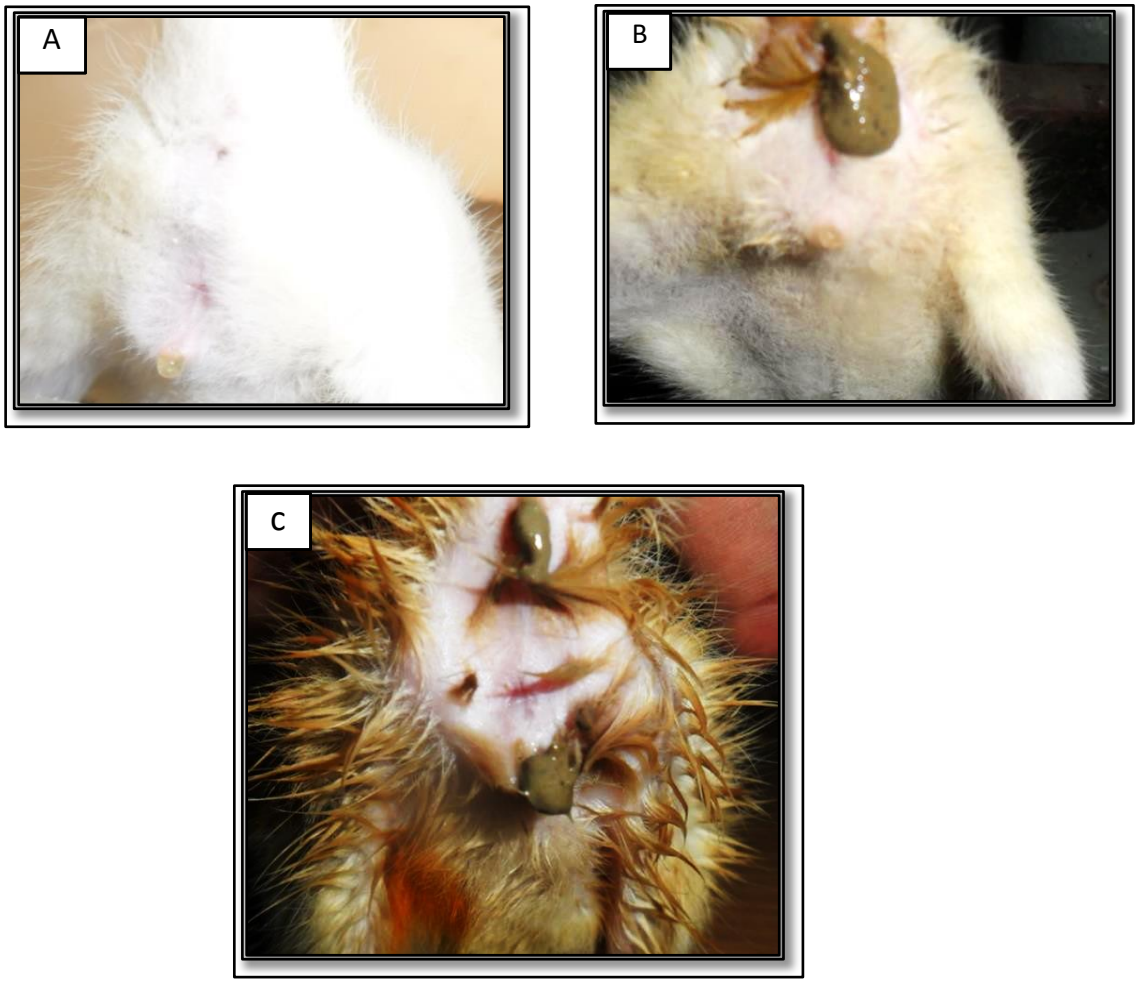


Disease Activity Index as recorded on 14th day of experiment

Values are expressed as Mean ±SEM; n=8. One-way ANOVA followed by pot-hoc Dunnett's test and Bonferroni's test.

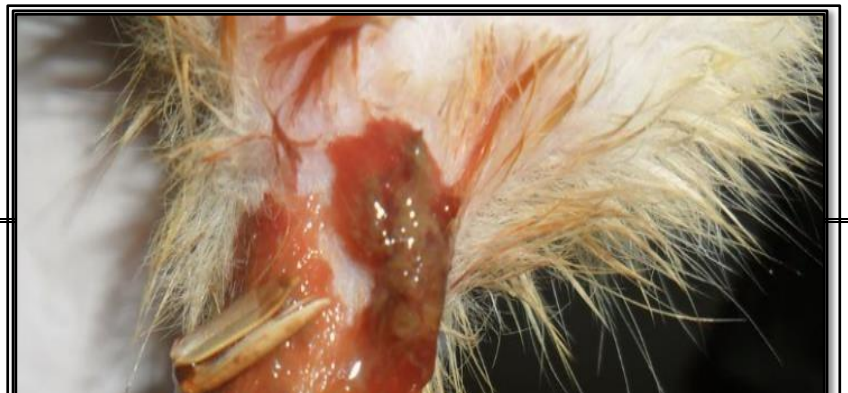
***p<0.05, **p<0.005 in comparison with Colitis-control group.**

Figure 11: Photographs showing status of diarrhoea before and after the induction of colitis.



A. Normal, B. diarrhoea with semi-formed stools, C. loose stools sticking to the peri-anal region.

Figure 12: Photograph showing the appearance of blood in stools after the induction of colitis.

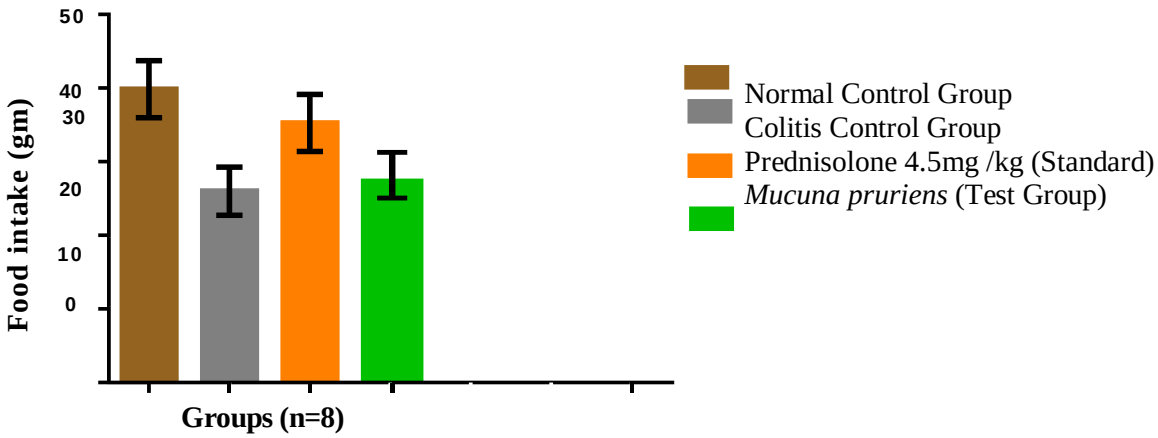


Food Intake

Food intake for all the animals was measured on alternate days from day 2 to day 14. There occurs no difference in food intake significantly among all groups.

Although, a definite trend seen. Animals with higher degree of colitis features has shown decreased food intake, and the intake has increased with the drugs showing good efficacy.

Graph 5: Effect of different treatment groups on food intake.



Values are expressed in Mean± SEM, n=8. There was no significant difference amongst the groups

INFLAMMATORY MARKERS

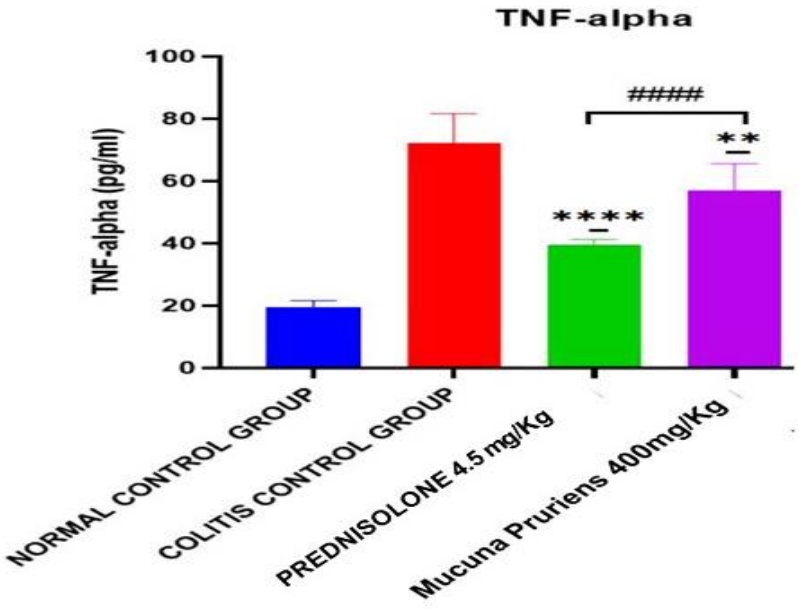
Serum TNF- α , IL 1-β, IL-6 levels were measured at the end of the study.

1. **Tumor Necrosis Factor – α**

At the end of the study the mean TNF-α values of normal control, Colitis control, prednisolone (Standard) , *Mucuna pruriens* (test) group were (15.92±1.800 , 87.45±4.836 , 41.57±1.789, 63.88±3.908) respectively. According to post-hoc Dunnett's test showed that the TNF-α in Colitis-control group was predominantly high compared to prednisolone group ($p < 0.0001$) and *Mucuna pruriens* ($p< 0.001$) . Bonferroni's analysis revealed that

the prednisolone group had significantly low TNF-α as compared to *Mucuna pruriens* group (p=0.0001).

GRAPH 6: TNF-α LEVEL AT THE END OF THE STUDY



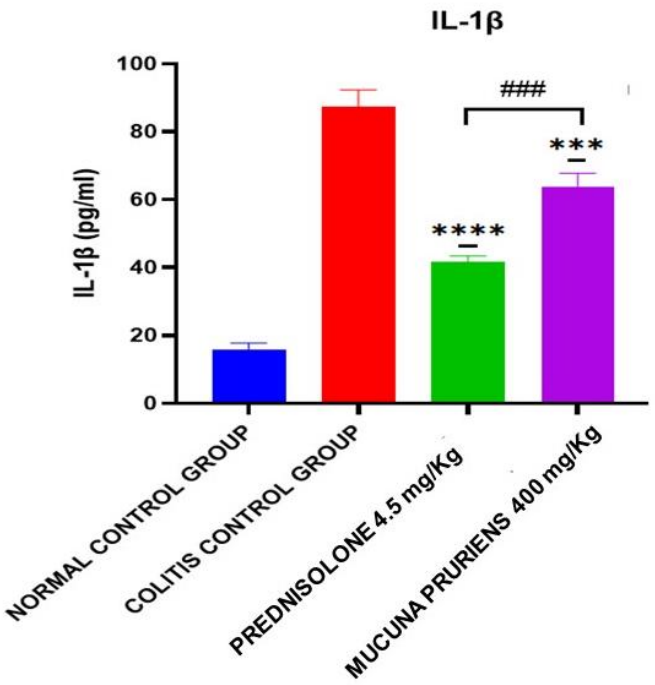
Values are expressed as Mean± SEM, n=8. One-way ANOVA followed by post-hoc Dunnet's test and Bonferroni's test.

******- p<0.0001; **- p= 0.001; ####- p=0.0001**

2. Interleukin 1-β

At the end of the study the mean IL 1-β values of normal control, Colitis control, prednisolone (Standard) , *Mucuna pruriens* (test) group were (20.25±4.270, 80.92±3.791, 25.43±1.857, 44.47±3.243) respectively. The post-hoc Dunnett's test showed that the IL-β value of the Colitis control group was significantly high compared to prednisolone group ($p < 0.0001$) and *Mucuna pruriens* ($p< 0.001$). According to Bonferroni's analysis the prednisolone group showed significantly low IL 1-β compared to *Mucuna pruriens* group (p=0.001).

GRAPH 7: IL 1-β LEVEL AT THE END OF THE STUDY.



Values are expressed as Mean± SEM, n=8. One-way ANOVA followed by post-hoc Dunnet's test and Bonferroni's test.

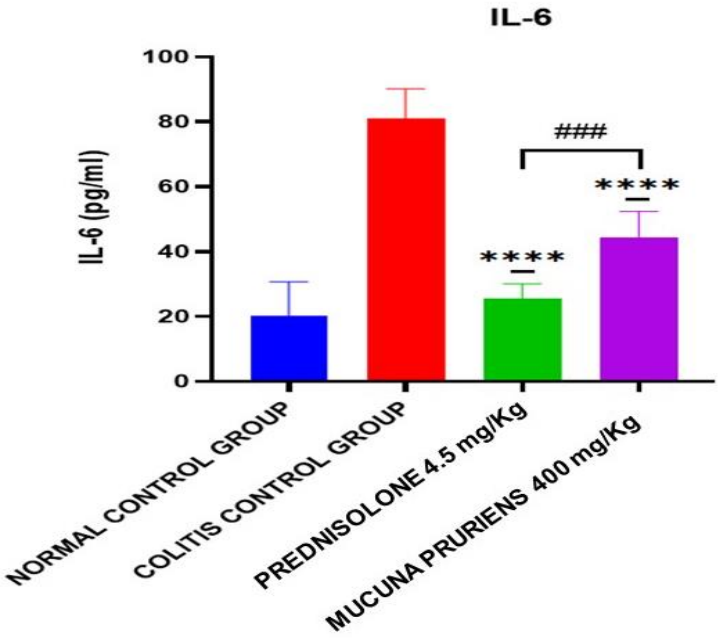
******- p<0.0001; ***- p<0.0001; ###- p=0.001.**

3. Interleukin- 6

At the end of the study the mean IL-6 values of normal control, Colitis control, prednisolone (Standard), *Mucuna pruriens* (test) group were (19.48±0.8639 , 72.17±3.896, 39.38±0.7648, 56.97±3.499) respectively. The post-hoc Dunnett's test demonstrated that the value of Colitis control group was significantly low compared to prednisolone group ($p < 0.0001$) and

Mucuna pruriens ($p< 0.001$). Post-hoc Bonferroni's analysis revealed that the IL-6 level was significantly low in prednisolone group compared to *Mucuna pruriens* group ($p=0.001$).

GRAPH 8: IL-6 LEVEL AT THE END OF THE STUDY



Values are expressed as Mean± SEM, n=8. One-way ANOVA followed by post-hoc Dunnet's test and Bonferroni's test.

******- p<0.0001; ###- p=0.001.**

TABLE 9: EFFECT OF VARIOUS TREATMENTS ON SERUM INFLAMMATORY MARKERS.

Inflammatory Cytokines (pg/ml)	Normal	Colitis control	Prednisolone 4.5 mg/kg	*Mucuna pruriens*
TNF-α	15.92±1.800	87.45±4.836	41.57±1.789 **** ####	63.88±3.908 **
IL-1β	20.25±4.270	80.92±3.791	25.43±1.857 **** ###	44.47±3.243 ***
IL-6	19.48±0.8639	72.17±3.896	39.38±0.7648 **** ###	56.97±3.499 ****

Values are expressed as Mean± SEM, n=8., * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, indicates the significant difference of various groups compared to colitis control by using ANOVA followed by post hoc Dunnett's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$, indicates the significant difference of Mucuna pruriens compared to prednisolone group done by using post hoc Bonferroni's test.

Histopathological studies

On histopathological examination, *Mucuna pruriens* significantly decreased the disease in terms of crypt distortion & inflammatory infiltrates. Reports of all the study groups were mentioned in the table below.

Table 10: Histo-pathological findings and Grading of all groups

CODE	GROUPS	FINDINGS	GRADING	REMARKS
GROUP 1	Normal control	Normal colonic mucosa	0	Both the Standard group (prednisolone) and test group *(Mucuna pruriens)* showed better scoring Histo-pathologically in comparison to Colitis control group.
GROUP 2	Colitis control	Marked inflammatory infiltrates with atrophy of mucosa	4	
GROUP 3	Standard-Prednisolone (4.5mg/kg)	Mild inflammatory infiltrates with almost normal crypts	0 +/- 1	
GROUP 4	*Mucuna pruriens*	Mild mucosal infiltrates and crypt distortion.	2	

Histo-pathological findings in various groups

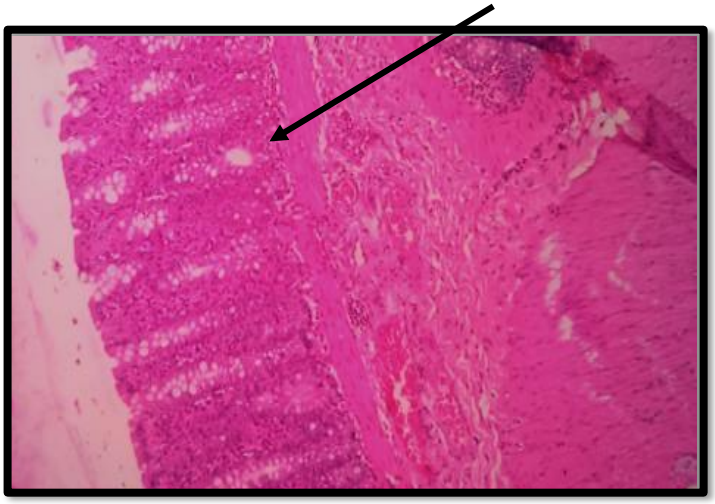


FIGURE 13: Normal Group showing the goblet cells

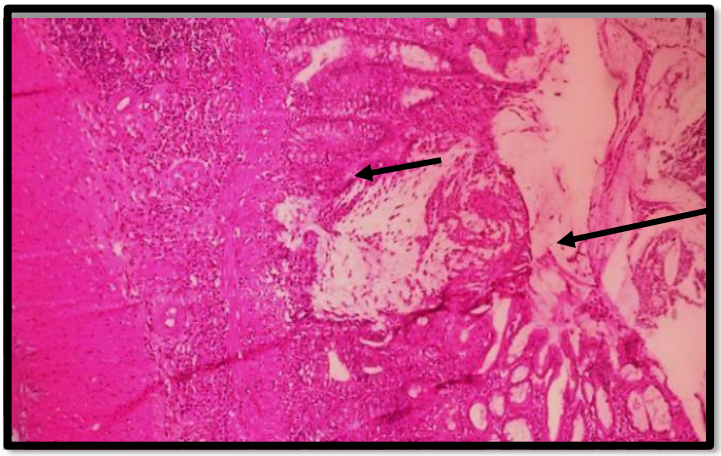


FIGURE14: Colitis control group showing Crypt distortion and destruction of villi

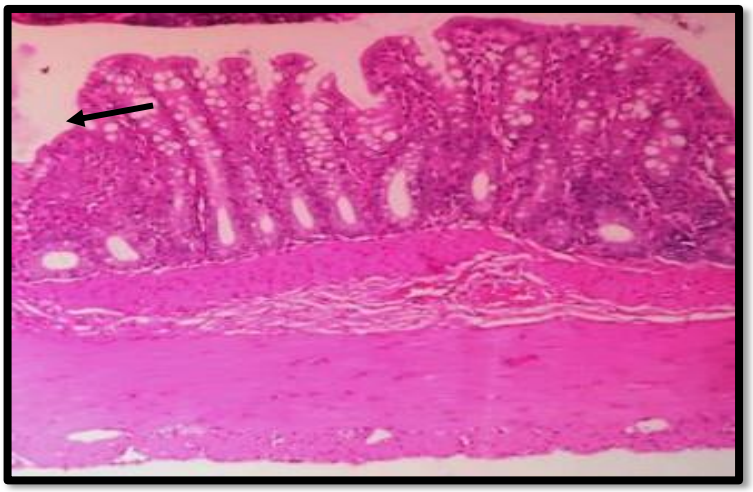


FIGURE 15: Standard Group – Prednisolone (4.5mg/kg) showing mild inflammatory infiltrates and normal crypts.

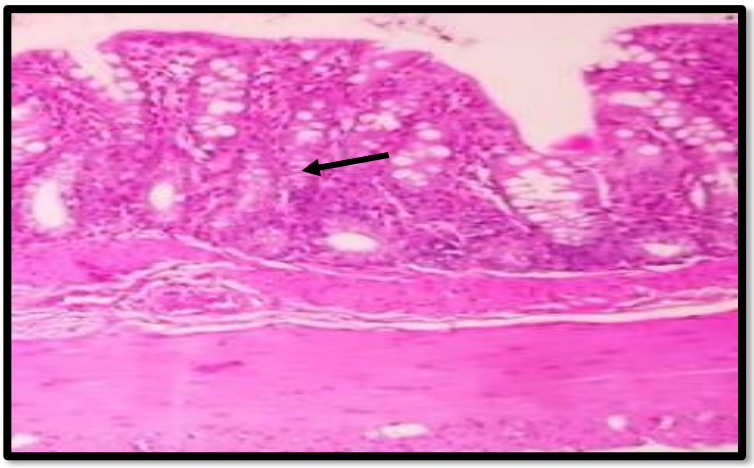


FIGURE 16 : Test Drug Group- *Mucuna pruriens* showing mild inflammatory infiltrates with mild distortion in crypts.

DISCUSSION

In the current study, the effect of *Mucuna pruriens* was estimated on TNBS-Induced experimental colitis in male Wistar rats. In terms of body weight, *Mucuna pruriens* had significantly prevented the weight loss when compared to Colitis control group. Disease Activity Index which is the most important indicator for the assessment of severity of colitis. At the end of the study, *Mucuna pruriens* reduced DAI significantly compared to Colitis control group. The reduction in DAI scores between prednisolone group and *Mucuna pruriens* group was comparable. The test drug *Mucuna pruriens* showed significant reduction in the IL 1-β, IL-6 and TNF-α. *Mucuna pruriens* showed better scoring histopathologically in comparison to Colitis control group.

Ulcerative colitis, which is a subcategory of inflammatory bowel disease affecting the colon has been termed as a lifestyle disease whose incidence and prevalence has increased now due to "westernization of lifestyle". [135] The same can be seen for a developing country like India, although there is lack of epidemiological data as only a few studies have been conducted until now. The pathogenesis is multifactorial, involving genetic predisposition, dysregulated immune responses, epithelial barrier defects and environmental factors. The aetiology, though not fully established, points towards multi-factorial causation involving genetic factors, environmental factors, immune dysregulation and gut microbiota.[136] Thus, with limited understanding of aetiology, the disease remains largely untreatable requiring lifelong medication.

Current pharmacotherapy is mainly focused on preventing the progression of the disease. Drugs like steroids, 5-ASAs, immunomodulators and biological agents are commonly used to maintain the patient in a state of remission. Each class of drugs has its own disadvantages. The current study is an attempt to find a safe alternative for the treatment of IBD.

Prednisolone reduces inflammation by inhibiting polymorphonuclear leukocyte movement and reversing enhanced capillary permeability. Therefore, it is first line drug in the treatment of Ulcerative Colitis.

Mucuna pruriens (Fabaceae) is a widely used herbal drug in ayurvedic medicine for the treatment of infertility in males, nervous system disorders.[137] In Ancient Indian medicinal system and Ayurvedic medicine, *Mucuna pruriens* seed extract are used for the several

inflammatory disorders. Previous studies revealed that *Mucuna pruriens* exhibit a variety of pharmacological effects, including anti-diabetic, neuroprotective, anti-inflammatory and anti-oxidant properties.[138] *Mucuna pruriens possess anti-inflammatory property which is mediated by inhibition of prostaglandin synthesis.*[138,139]

TNBS model is the most commonly used paradigm for experimental colitis. It provides most effective reproducible method of induction of colitis which closely mimics the Ulcerative Colitis in humans. In this study, Colitis-control group has revealed predominant difference from Normal group from day 4 of induction of colitis to the final day of study. It indicates the accurate induction of Colitis & disease activity state.[139]

Effect on body weight:

In the current study, the body weight of all the groups were compared with the colitis control group from day 1 to day 14. After the induction of colitis there was significant reduction in body weight. Both the treatment groups prednisolone (4.5mg/kg) and *Mucuna pruriens* significantly prevented weight loss when it is compared to colitis control group.

A study done by Olugbenga David Oloruntola et al. suggested that there was significant weight gain after the administration of Mucuna leaf meal (MLM) for 7 days on rabbits which supports our study.[140] Another study done by James O. Daramola et al on West African dwarf bucks suggested that there was significant improvement in body weight after the administration of *Mucuna pruriens* seed powder 75mg for 30 days perorally and gained weight at the end of the study which also supports our present study. [141] Furthermore, A study demonstrated by Ani AO et al. on pullet chicks administered with Mucuna seed meal (MSM), which shows that there was significant increase in weight gain of 15- 20 percent after the intake of *Mucuna pruriens* seeds) was supported with the current study.[142]

Effect on disease activity index:

DAI is the most accurate and important factor for determination of severity of Colitis. DAI scores were evaluated primarily on body weight change & clinical signs including faecal blood and irregular stool consistency. The higher DAI scores indicates more severe Colitis. In our study, Disease Activity Index was compared with the Colitis control group on day 1, 7, and 14 day.

On day 1 the DAI score revealed no significant difference amongst the groups. DAI scores Day 7 found to have significant difference between Colitis-control & standard drug group, resembling the favourable change to the standard treatment. DAI values on day 14 significantly reduced in *Mucuna pruriens* group when it was compared to colitis-control group. DAI on day 14 between prednisolone group and *Mucuna pruriens* group was comparable.

In our study, we found that prednisolone produce weight gain in experimental colitis model which is supported by the clinical study done by Paul Rutgeerts et al. on inflammatory bowel disease in patients. The dose of prednisolone was 40 mg per day in a controlled-released tablet form for two weeks, after which it was gradually reduced to 5 mg per day during the last week. In this trial it was observed that prednisolone reduced the score of Disease Activity Index.[143]

Effect on Inflammatory Markers:

Pro inflammatory cytokines (e.g., TNF-α, IL-1β and IL-6) released from macrophages, neutrophils and endothelial cells are excessively produced in TNBS-induced colitis. Interleukin 1 beta, Interleukin 6, TNF -alpha are most important parameter of the inflammatory response. The test drug *Mucuna pruriens* showed significant reduction in the IL 1 Beta, IL-6 and TNF- alpha. These findings of the study are supported and done by another clinical study Aungkana Rachseea et al. to determine effect of *Mucuna pruriens* seed extract. Inflammatory mediators levels were analyzed by using ELISA test. The study concluded that *Mucuna pruriens* seed extract shows significant effect in inhibiting the release of inflammatory mediators including nitric oxide (NO), IL-1β, IL-6, and TNF-α.[144]
Mucuna pruriens herbal plants shows the anti-oxidant and anti-inflammatory properties. The drug also exhibits and shows positive result in neuroinflammation.

Another clinical study done by Sachchida N. Rai et al is supported with the present study. The study found out the effect found in aqueous extract *Mucuna pruriens* produced by neuro-inflammation, which was administered orally to mice showing significant improvement in inflammatory mediators like Tumor Necrosis Factor alpha, Glial Fibrillary Acidic Protein, Intercellular Cell Adhesion Molecule, and Inducible Nitric Oxide Synthase in substantia nigra of mice in Parkinson's disease.[145]

Mucuna pruriens, an herbal drug is the most widely used drug in Ayurvedic medicine. Several studies have reported that it possesses analgesic, anti-neoplastic, anti-microbial,

anti-inflammatory and anti-epileptic properties. Hiam Elabd et al. reported that the nutritional effects present in *Mucuna pruriens* seed extract in growth performance, hepatic function, status of immunity, gonadal histology, biochemical profiles and expression of immune-related genes in mono-sex Nile tilapia fish (Oreochromis niloticus). The study concluded by showing significant reduction in interleukin 1-β (IL-1β after administration of *Mucuna pruriens*.[146]

Another study done by Martínez Leo et al. investigated whether proinflammatory mediator production by BALB/c mice macrophages was affected by the enzyme action of protein derivatives from Mucuna pruriens L. showing significant reduction in TNF alpha and IL- β.[147] The present study showed significant reduction in interleukin β, and TNF alpha after the administration of *Mucuna pruriens* in rat. The study done by Roberto Edén et al. stated to find out the effect of immunosuppressive from protein derivatives of Mucuna pruriens on a murine model of Type 1 diabetes mellitus. The study showed significant reduction in inflammatory mediators interleukin β and TNF alpha.[148] In our study after administration of prednisolone there was significantly reduction in inflammatory markers which was supported by the study by Trent Woodruff et al in inflammatory bowel disease where they used TriNitrobenzene sulfonic acid (TNBS)- to induced colitis in rats. The study concluded that prednisolone (1 mg/kg/day subcutaneously) showed significant improvement in inflammatory mediators.[149]

HISTO-PATHOLOGY

Colon: At the end of our study, the rats were sacrificed and colon was dissected out for histopathological examination. The colon was histopathological graded with a grading score of 1 to 4 to determine the severity of the colitis. (Grade1 indicates milder form of Colitis, Grade 4 indicates severe form of Colitis).

On histopathological examination, normal control group showed histopathological findings with normal colonic mucosa denoting grade 0; Colitis control group showed marked inflammatory infiltrates with atrophy of mucosa denoting grade 4; Standard (prednisolone) group showed mild inflammatory infiltrates with normal crypt distortion denoting grade 0 or ± 1; Test (*Mucuna pruriens*) group showed mild mucosal infiltrates and crypt distortion denoting grade 2. Both Standard group (prednisolone) and Test (*Mucuna pruriens*) group showed better scoring histopathologically in comparison to Colitis control group.

LIMITATIONS AND FUTURE RECOMMENDATIONS:

The effect of *Mucuna pruriens* had considerable impact on Disease activity index, Body weight and inflammatory parameters.

Strength of the study includes extension of the study to biochemical parameters to substantiate the physical and histopathological parameters.

This study has few limitations, viz; since IBD is a chronic illness route of drug administration should have been intraperitoneal than oral, but many studies were done with parenteral route also, and myeloperoxidase level is an important and should be included in this study.

CONCLUSION

In the current study, *Mucuna pruriens* is used to explore the beneficial outcomes of experimental colitis in wistar male rats. TNBS is employed to induce experimental colitis in male Wistar rats, which is a widely used and recommended method for investigating inducible experimental colitis.

Present study, concluded *Mucuna pruriens,* decreases the symptoms of Colitis and pro-inflammatory cytokine level markers like TNF-α, IL-6 and IL-1β in TNBS induced colitis in male Wistar rats.

In conclusion, this study has showed the beneficial effect of *Mucuna pruriens* in experimental colitis. More studies, both clinical and pre-clinical should be planned to corroborate the evidence presented in this study.

SUMMARY

Current study was planned to explore the alternative treatment possibilities for ulcerative colitis, a type of inflammatory bowel disease. IBD is on rise currently, more so in developing countries like India due to increasing westernization. Thus, this study was conducted with the following objectives:

1. To determine effect of *Mucuna pruriens* on Trinitrobenzene sulfonic acid model of experimental colitis in Wistar rats.
2. To determine the effect of *Mucuna pruriens* on pro-inflammatory Cytokines (TNF-α, IL-1β, IL-6).

The experimental colitis model used in this study was TNBS colitis. It is one of the most commonly used paradigm for experimental colitis. This model provides an easy, accurate and reproducible process which closely mimics ulcerative colitis in humans.

This basis for this study was rooted in previously proven role of Steroids in gut inflammatory process. Prednisolone has been proven to promote inflammation through the release of neuropeptides like substance P, prostaglandin and cytokines. Thus, paving the way for studying anti-inflammatory activity.

Results from the present study states that Prednisolone and *Mucuna pruriens* effectively reduce the symptoms, and pro-inflammatory cytokine levels in TNBS induced colitis in Wistar rats.

The, present study recommends that more clinical and pre-clinical trials should be planned to further enhance the adoption of these drugs into clinical practice as the currently available therapy is inadequate.

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ANNEXURE

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed to be University)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI – 590010, (KARNATAKA).
INSTITUTIONAL ANIMAL ETHICS COMMITTEE.
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Main Nominee – CPCSEA
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Central Animal House,
JNMC, Belagavi.

Prof of Anatomy,
JNMC,Belagavi

Link Nominee CPCSEA.
Dept of Pharmacology &
Toxicology
KLE's Coll Of Pharmacy,
Hubballi

CERTIFICATE

This is to certify that the M.D/ M.D.S/ Ph.D/ Research project Entitled " Effect of *Mucuna pruriens* seed extract on Tri Nitro Benzene Sulphonic Acid (TNBS) model of experimental colitis in male Wistar rats- An experimental study".

Submitted by- PG Pharmacology, JNMC.

Has been approved by the Institutional Animal Ethical Committee Meeting held on 5.2.2021 vide Resolution No. 14/3.

For sanction of 8 Male Wistar Rats

(Dr. P.A Patil)

Signature and Name:
CPCSEA-Main Nominee
Main Nominee CPCSEA
IAEC-JNMC, Belagavi.

Signature and Name:
Chairman/Mem.Secretary
Member Secretary
IAEC-JNMC, Belagavi.