CHAPTER 2

RESEARCH ENVISAGED
2.1. BACKGROUND

Diarrhea is mainly caused by abnormal fluid and electrolyte transport, decreased absorption or increased secretion in the small and large intestines. The allopathic treatment for diarrhea includes administration of oral rehydration solutions to replace the lost fluids and electrolytes and drugs such as anti-secretory, anti-motility and antispasmodic to mitigate the on-going fluid loss and the associated pain. Antimicrobial therapy is used in case of infectious diarrhea. At present the therapy used currently is associated with certain side effects such as constipation, drug addiction, drug toxicity and antimicrobial resistance. Thus there is need for newer therapeutic drugs with higher efficacy and lesser or no side effects.

Diarrhea is a prevalent symptom and sign of patients with inflammatory disorders of the intestines including microscopic colitis and IBD. The current therapeutic strategy used in treating inflammatory disorders of the intestines includes drugs to reduce the inflammation and the symptomatic treatment to reduce the associated symptoms. Inflammation is reduced by administration of drugs such as anti-inflammatory drugs, immunosuppressants, biologic agents, antibiotics and probiotics. The associated symptoms including diarrhea is relieved by administration of antisecretory, antimitility and antispasmodic drugs and pain is relieved by administration of drugs such as antidepressants, anticonvulsants and opiates. The drugs used to reduce inflammation in inflammatory disorders of the intestines have several side effects and some of the drugs in turn aggravate diarrhea. Also there are no specific therapies for treating pain in inflammatory disorders of the intestines. Many patients use NSAIDs for pain management because they are available in over-the-counter formulations. The use of NSAIDs would aggravate the inflammation further and even worsen the inflammation.

Hence there is a need to develop a holistic treatment for treating inflammatory disorders of intestine and the associated symptoms including diarrhea and pain.

PUD is commonly caused by H. pylori bacteria and NSAIDs. The mortality rate of PUD has fallen, but because of the widespread use of NSAIDs and low-dose aspirin the economic burden of PUD had remained significant. Also there is increase in the proportion of idiopathic PUD and the recurrence rate is significantly higher than NSAIDs
and *H. pylori* induced peptic ulcers.\(^{36,37}\) As the prevalence of NSAIDs-induced ulcers is increasing than that of *H. pylori*-induced ulcers, a number of pharmaceutical companies are now developing combination medications of NSAIDs and PPIs to target NSAID-induced peptic ulcers.\(^{77}\) Thus considering the recent trend in PUD market and the prevalence of PUD, there is a need to develop a treatment with antiulcer effect.

Herbal medicines have the potential to provide such holistic treatment, as they contain many phytoconstituents such as phenolics, alkaloids, tannins, flavonoids which provide the additive or synergistic effect by acting on the different molecular targets involved in pathogenesis. Herbal medicines treat symptoms and address the disharmony produced by the underlying disease thus treating the disease at a grass root level and with relatively lesser/no side effects.\(^{78}\)

The demand for the herbal medicines among patients is also rising because of the faith that herbal medicines are effective, safe and are more accessible. But at present, a major hindrance in the use of herbal medicines is the lack of scientific and clinical data proving their efficacy and safety. Thus there is need to validate the efficacy of herbal medicines scientifically in order to enjoy its maximum benefits. For herbal medicines to be perceived at the global level, there is a need to ensure the safety, efficacy and quality control of herbs and herbal formulations via the use of modern techniques such as proper botanical identification of all medicinal plants; processing those plants in a scientific, economic and safe manner using methods similar to the methods used for modern drugs; chemical characterization of active and inorganic constituents; pharmacological studies of each plant to ascertain efficacy and safety; standardization to ensure uniformity and finally documentation of the research.\(^{73}\)

The plants selected for this study were based on the evidence of the ethnopharmacological usage, sustainable use of the plant and availability of the plant.

### 2.2. AIMS AND OBJECTIVE

Mustard seeds are used as spice and traditionally it is used in various stomach ailments. The therapeutic benefits of mustard seeds in GI disorders including diarrhea, inflammatory disorders of intestine and PUD are not explored. Thus, it is anticipated to
explore the biological benefits of mustard seeds in these GI disorders and subjected it to detailed scientific investigation.

*Punica granatum* fruit peel (PGFP) is known for its therapeutic benefits since ancient time in GI disorders. Few studies are performed on PGFP to prove the therapeutic benefits in diarrhea and PUD. Detailed scientific investigation will add more credentials to its usage. From the environmental prospective, the PGFP is enormously produced byproducts by agro-food industry. It can be reused for its therapeutic activity as it contains many bioactive molecules.

Considering the above scenario, the aim of the present study was to carry out the phytochemical and pharmacological investigations of Mustard seeds of two different varieties-*Brassica nigra* and *Brassica juncea* and *Punica granatum* fruit peel for antidiarrheal, anti-inflammatory, analgesic and antiulcer activity.

The objectives of the study were,

1. Screening of plant extracts for treatment in Inflammatory bowel diseases and its associated symptoms
   a. Diarrhea
   b. Colitis
   c. Pain
2. Screening of plant extracts for the treatment in gastric ulcer (Peptic Ulcer Diseases)
3. Development of suitable dosage forms containing plant extract/s.
4. Pharmacological evaluation of developed formulation/s.
5. Standardization of developed formulation/s

**2.3. PLAN OF WORK**

1. Literature survey and review of literature
2. Selection, Procurement and Authentication of plant materials and its standardization
3. Preparation of plant extracts
4. Phytochemical screening of plant extracts using qualitative tests
5. Screening of extracts by *in vitro* antioxidant assays:
   a. DPPH radical scavenging activity
   b. Nitric oxide radical scavenging activity
6. Screening of extracts by *in vitro* antimicrobial assays:
   a. Ditch Plate method
   b. Cup Plate method
7. Screening of extracts by *in vitro* anti-inflammatory assays:
   a. Human Red Blood Stabilization method
8. Screening of extracts by *in vitro* anthelmintic assay
9. Acute toxicity studies of bioactive extracts
10. Evaluation of *in vivo* antidiarrheal activity of bioactive extracts by using following animal models:
   a. Castor oil induced diarrhea
   b. Charcoal meal test
   c. Enteropooling assay
11. Evaluation of *in vivo* anti-inflammatory activity of bioactive extracts by using following animal models:
   a. Carrageenan Induced paw edema
   b. Trinitrobenzene sulphonie acid induced colitis
12. Evaluation of *in vivo* analgesic activity of bioactive extracts by using following animal models:
   a. Acetic acid induced writhing test
   b. Hot Plate method
13. Evaluation of *in vivo* antiulcer activity of bioactive extracts by using following animal models:
   a. Ethanol induced ulcers
   b. Aspirin induced ulcers
   c. Stress induced ulcers
14. Formulation and evaluation of suitable oral dosage formulation/s containing bioactive extract/s.
15. Evaluation of *in vivo* antidiarrheal activity of developed formulation/s by using following animal models:
   a. Castor oil induced diarrhea
   b. Charcoal meal test
c. Enter pooling assay

16. Evaluation of *in vivo* anti-inflammatory activity of developed formulation/s by using following animal models:
   a. Carrageenan induced paw edema
   b. Tri nitrobenzene sulphonyl acid induced colitis.

17. Evaluation of *in vivo* analgesic activity of developed formulation/s by using following animal models:
   a. Acetic acid induced writhing test
   b. Hot Plate method

18. Evaluation of *in vivo* antiulcer activity of developed formulation/s by using following animal models:
   a. Ethanol induced ulcer
   b. Aspirin induced ulcer
   c. Stress induced ulcer

19. Standardization of developed formulation/s using bioactive marker/s by HPLC studies.