ROLE OF AYURVEDIC GRAHANIROGA TREATMENT PROTOCOL IN THE MODULATION OF GUT MICROBIOTA IN IRRITABLE BOWEL SYNDROME- A CLINICAL TRIAL

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List of Abbreviations: IBS-M (Irritable Bowel Syndrome-Mixed), FGID (Functional Gastrointestinal Disorder), VAS (Visual analogue scale), IBS-SSS (IBS-Symptom severity scale), IBS-QOL (Quality of life questionnaire), BSF (Bristol stool form scale) CCB (Calcium channel blockade), GIT (Gastrointestinal tract)

Conflict of Interest: None Declared.

Keywords:

Gut microbiota, *Grahaṇi*treatment protocol, Irritable Bowel syndrome-Mixed, *Priyaṅguambaṣṭādicūrṇa*, Buttermilk.

Highlights and Novelty of the Study:

- The study clearly demonstrates the importance of Gut dysbiosis in the pathoph ysiology of irritable bowel syndrome.
- The Mahasrotas (GIT) and its homeostasis are crucial for maintaining Gut health, according to Ayurvedic research.
- The Grahaniroga therapy procedure employed here shows promise against every evaluation criterion.
- So far, no Ayurvedic study has been conducted to explore the role of Grahani Treatment protocol in the Gut Regulation and Modulation.
- This study can be considered as "**First of its kind**", proving its novelty.

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Abstract:

Background and Aim:

IBS is a FGID, characterised by abdominal pain linked to changes in stools' frequency and/or form. Inability to predict symptoms, embarrassment, depression, and self-consciousness are all commonly seen, with a frequency of 15% among all Indians and 11.2% worldwide. Alterations of the gut flora in IBS-M causes change in stool pattern. Pathophysiology is linked to dysbiosis, and among the strains, individuals with IBS-M have decreased Bifidobacterium and Lactobacilli.The treatment plan involves dietary modifications, medicine, probiotics, and psychological support.IBS-M, is similar to the *Lakṣanas* of *Grahaṇiroga* resulting due to the impairment of *Agni*.

Experimental procedure:

A Pre and Post-testclinical study with 10 patients fulfilling the Inclusion and Exclusion Criteria.

Results and Discussion:

Outcome variables included BSF, IBS-SSS, VAS, IBS - QoL and Gut assessment. Assessment was done on the 0^{th} and 45^{th} day. Statistical analysis was done by Paired t-test. The results were statistically significant (p value <0.05).

The protocol containing*VacāharidrādhiCūrṇa*being *ĀmaAtisāraghna*, and *DāḍimādiGhṛta*being *MūḍaVātānulomana* corrected the *Mandāgni*. *Virecana*acted by its *Indriyaprasāda* and *Pittahara* property and *Priyaṅguambaśtādi*Cūrṇaacted by its *Pakvātisāraghna*and *PurīṣaSaṃgraha* property. The study drugs aided in reduction in VAS scoreby its anticholinergic/CCB property. Intestinal motility was modified by anti-secretory effects. Finally, the Antibacterial activity of the drugs against S.aureus and E.coli and*Takras's* probiotic activity modulated the Dysbiosed Gut.

Conclusion:

Thus, the *Grahaṇiroga* Treatment Protocol modulated the Gut Microbiota and was alsoeffective in reducing the signs and symptoms of IBS-M.

1.1 Introduction:

The term "irritable bowel syndrome" (IBS) refers to a functional disorder of the gastrointestinal tract that does not have any accompanying structural defects and in which the normal function of the bowel is either exaggerated or distorted in a way that frequently results in constipation, diarrhoea, and/or abdominal pain or discomfort. IBS affects between 10% and 25% of the world's population, with a prevalence of 15% among India's general population. IBS-Mixed is more common, with a 42.4% prevalence, and its challenging to treat because of the alternating or mixed stool pattern.[1,2]

People who have IBS usually describe feeling down, embarrassed, self-conscious, and unable to predict symptoms, which significantly complicates their daily life.

The illness has a detrimental impact on interpersonal interactions and restricts inv olvement in daily social activities. Many IBS sufferers feel that they are not taken seriously and that being diagnosed with the condition can make them feel stigmati sed. Some people with IBS could be deterred from seeking medical attention beca use they think the HCPs (Health Care Professionals) won't listen to them, and a la ck of adequate assistance may further exacerbate feelings of social isolation. Acco rding to studies, IBS accounts for 20-50% of all patient referrals to gastroenterologists, making it the most common cau se of referral.[3]

IBS is a complicated pathophysiology since it is influenced by a wide range of factors, including psychological ones, changes in gastrointestinal motility, aberrant visceral perception, and food sensitivity. The idea that IBS symptoms are caused by dysregulation of the "brain-gut axis," which shows up as increased visceral perception, is a common thread. Dysbiosis, or the disruption of the physiologic symbiotic relationship (eubiosis) between the human host and the microbiota, is thought to be the primary cause of IBS in the majority of patients.Current research suggests that IBS is actually a disorder of the microbiota and the GBA (Gut Brain Axis) since dysbiosis is seen in IBS, and the immunological response that results may exacerbate and perpetuate the condition's gastrointestinal symptoms. Western nations are known to have a higher prevalence than Asian nations.[1,4]

Clinical diagnosis of Irritable Bowel Syndrome is made using the ROME-IV criteria for IBS, and biochemical confirmation is made by a shift in the gut microbiota. Treatments for IBS differ from one person to another because it is a biopsychologic condition involving irregular motility and altered visceral sensations. Antispasmodics, antidiarrheal medications, and antidepressants are some of the well-established treatments used in the traditional therapy of IBS. Prebiotics and probiotics are a few new treatments, but they are not firmly planted in routine clinical practise.[3]

*Agni*is the invariable agent in the process of *Āhārapāka*[5](digestion, transformation) and *ĀcāryaVāgbhaṭa*has mentioned that the root cause of every disease is‘*Mandāgni*’.[6] An Ayurvedic concept known as *Grahaṇi*[7] refers to the location of Agni, or the digestive fire, which aids in food metabolism and digestion. One of the most common diseases of the gastrointestinal

system,*Grahaṇiroga* is one that is frequently observed in daily clinical practise. *Mandāgni*produces *Āmadoṣā,* which eventually might lead to *Grahaṇiroga*.*Ācārya Caraka* says that *Grahaṇiroga* and *Agni* are interdependent, functionally weak *Agni* i.e., *Durbala Agni*, leads to partial digestion and partially undigested bio substances which moves downward in gastrointestinal tract, produces a disorder known as *Grahaṇiroga*.[8]Most ailments have their roots in *Āmadoṣā*, which is a result of *Durbala Agni* and in the pathophysiology of *Grahaṇiroga*, it plays a pivotal role. According to *ĀcāryaVāgbhaṭa,Grahaṇiroga* is considered has one of the eight major diseases (*AṣṭaMahāgada*).[9]

The unique concept of *SahajaKṛmi*was first described in *CarakaSamhitā,* causes *ŚarīraDhāraṇa* (supports body) and it is mentioned along with the other type of*Kṛmi*which are *Avaikārika* (Non-pathogenic). *VaikārikaKṛmi* (Pathogenic) are twenty in numberand rest all comes under *SahajaKṛmi*, inferring they are innumerable.The term "*Sahaja*" here refers to organisms that are commensal to the body, i.e., the body feeds the development of the intestinal microbiota (*SahajaKṛmi*). *SahajaKṛmi* holds out a variety of functions that support the body and maintain health, which is similar to the modern concept of Gut Microbiota.[10]

2. Materials and Methods:

The present study design was a single arm pre and post-test study, to assess the efficacy of Grahani treatment protocol on the signs and symptoms of IBS-M and its influence on Gut Microbiota flora in IBS-M patients.

2.1. Patient Recruitment and Data collection:

Patients from the OPD and IPD of Amrita Ayurveda Hospital, Vallikavu, Kollam, Kerala under the postgraduate department of Kāyachikitsa

with symptoms of Irritable Bowel Syndrome were initially screened using ROME-IV criteria for IBS and Gut microbiota identification.

2.2. Inclusion criteria:

- Participants fulfilling ROME IV CRITERIA
- Age: 20 - 45 years with no discrimination of sex, caste, religion and economic status.
- Patients identified with the presence of specific strains of Lactobacilli and Bifidobacteria from their fecal samples.
- Participants from whom the written consent is obtained.

2.3. Exclusion criteria:

- Those with the endoscopic findings of peptic ulcer or any organic lesions and other systemic disorders
- Those on NSAID's in the last six months, antibiotics or other long-term and continuous medication
- Patients with alcohol dependency or drug dependency.
- Pregnant women and lactating mothers.

2.4. Diagnostic criteria:

Diagnosis was done based on Rome IV Criteria for Irritable Bowel Syndrome.Recurrent abdominal pain on average at least 1 day/week in the last 3 months, associated with two or more of the following criteria:

a. Related to defecation

b. Associated with a change in frequency of stool

c. Associated with a change in form (appearance) of stool.

Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

2.5. Assessment criteria:

Before and After assessment of the treatment was done i.e.on 0^{th} day and after 45 daysusing Bristol stool form scale, IBS-SSS(symptom severity scale), VAS (visual analogue scale), IBS – QoL (quality of life scale), Assessment of Gut Micro Flora in stool samples.

2.5.1.Biochemical Assessment:

Methodology of identification and quantification of Bifidobacterium and Lactobacillus species from Human Fecal Microbiota

a) Stool collection protocol:

The stool collection for gut microbiome study requires more consideration and modification. Fecal samples for studies on the gut microbiota should ideally be obtained in sufficient quantities to allow for aliquoting. To prevent contamination by toilet water and urine, the fecal samples are frequently collected in large quantities and carefully handled. Put the stool sample in a wide-mouth container or cover the toilet seat opening with a plastic bag or wrap. This prevents the stool sample from entering the toilet.A scoop is affixed to the vial cap of each collection vial. Use the included spoon to roughly quarter-fill the specimen pot with gloved hands. The faces must assume the shape of the container and may be liquid, formed, or semi-solid. Finally, since alcohol handrubs are useless against Clostridium difficile, wash your hands with soap and water to disinfect them. Stool collection, however, can be a laborious task for some of the study volunteers due to sanitary considerations and sensory reasons, and can occasionally generate feelings of humiliation.

Culture media preparation:

- Broth preparation: Suspended 6 g of Soyabean Casein Digest Medium

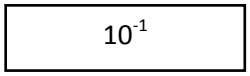
(M011-500g, Himedia) in 200ml MilliQ water as recommended by themanufacturer. Mixed well and dispense 5ml each sterile screw cap tubes.Sterilize by autoclaving at 15 lbs pressure, 121°C for 15 minutes in autoclave(Panasonic, US). Tighten the caps and keep refrigerated until use.

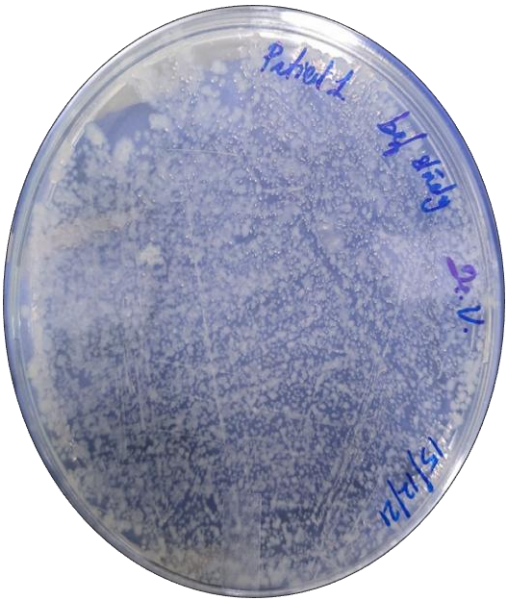
- Agar plate preparation: Suspended 6 g of Soyabean Casein Digest Medium (M011-500g, Himedia) in 200ml MilliQ water as recommended by the manufacturer. To this add 3g of Bacteriological Agar- Agar (Merck, Germany). Mixed well and sterilized by autoclaving at 15 lbs pressure, 121°C for 15 minutes in autoclave (Panasonic, US). Pour the autoclaved media aseptically into sterile petri dishes (1/3 of each plate) inside biosafety cabinet. Allow the media to solidify. Keep refrigerated until use.

b) Sample analysis:

- 1g of stool sample was taken and was serially diluted till 10-5. Each dilution was plated in media agar plates and incubated at 37°C for 24 hours in a sterile bacteriological incubator.
- The plates were analyzed and optimal dilution was foundout by counting the colonies grown.

Figure.1: Serial Dilution and Plating





10^{-3}

10^{-5}

Biochemical characterization of Bacterial strains:

The biochemical characterization test was conducted using the pure cultures of various bacterial strains. The Lactobacillus KB020 and Bifidobacterium KB021 rapid biochemical identification test kits, which include a variety of biochemical tests, were used. These identification kits had 12 different biochemical tests in total. They are Esculin Hydrolysis, Catalase, Xylose, Celluboise, Arabinose, Maltose, Galactose, Mannose, Mellibiose, Raffinose, Sucrose, and Trehalose.By removing the sealing tape, the test kits were opened in an aseptic manner. By using the stab inoculation approach, a loop of 24-hour-old bacterial cultures was placed in each test kit well before being sealed with sealing tape that had been torn off. The kits were kept at a temperature of 35–37°C for 24 hours. The tests were conducted using the pH change and substrate utilisation principles. When bacteria are introduced to test kit media, the substrate changes as a result of their metabolic activity. This is shown by a colour change in the medium, which can be seen visually, or by the inclusion of the appropriate test reagent aseptically after the incubation period.The standards mentioned in the result interpretation chart were used to

analyse the results. To get test results after the incubation time, aseptic reagent addition was performed as indicated.

2.6. Intervention:

Table No.1:- Therapeutic intervention with Duration

S.NO	**Treatment**	**Int. medicine**	**Ext. medicine**	**Dosage**	**Anupana**	**Duration**
1.	*Pācana-Dīpana*	*VachāharidhardhiKashaya*		50ml twice daily before food, orally	Warm water	3-7 days
2.	*Snēhapāna*	*DādimādiGhṛta*		50ml, 100ml and 150 ml empty stomach.		3 days
3.	*Sarvānga Abhyanga BāshpaSwēda*		*Tila Taila*			3 days
4.	*KōṣṭaSodana*	*Avipatti cūrna*		40 grams with Honey.		1 day

5.	*Samsarjana Krama*	According to *Śuddhi**				
6.	*Shamana Oushadha*	*Priyaṅguambaṣṭā digana cūrna*		6 grams twice daily before food	Takra	30 days

**PravaraŚuddhi*- 5 days; *MadhyamaŚuddhi*- 3 days; *Avara Śuddhi*-1 day.

3. Statistical Analysis:

The final conclusion was reached after statistical analysis of the results using the Paired t-test.

4. Results:

The effects of therapy on different assessment scales(both before and after treatment)are detailed in **Table No:2**.

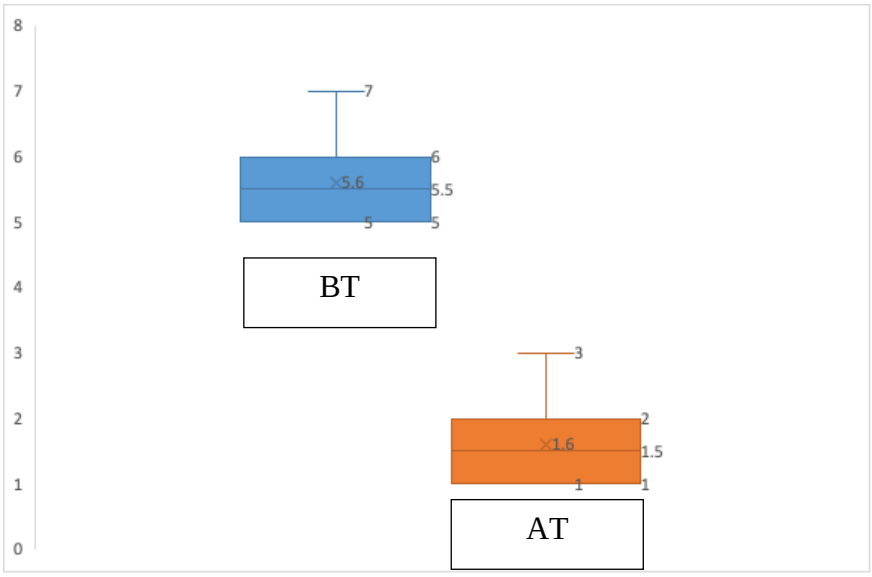
In the present study, 10 subjects of Irritable Bowel Syndrome-Mixed (IBS-M) were registered and completed the treatment protocol. The effects of therapy on various parameters of 10 subjects of IBS-M are presented under the separate headings. Paired t test was used for statistical analysis of the collected data.

Table No.2:- Effects of Therapy on Assessment criteria's

DOMAIN	**Mean**	**SD**	**Mean Difference**	**t-value**	**p-value**

VAS Scale	BT	5.6				
	AT	1.6	.6666	4	16.9737	<0.0001
Gut Microbiota	BT	145.9	55.7802		-3.3630	
	AT	253	60.395	-107.1		<0.05
IBS-SSS	BT	278.5	17.64621			
	AT	60.5	10.65885	218	50.7602	<0.0001
IBS-QOL	BT	99.3	5.3343			
	AT	57.4	9.6976	41.9	12.1809	<0.0001

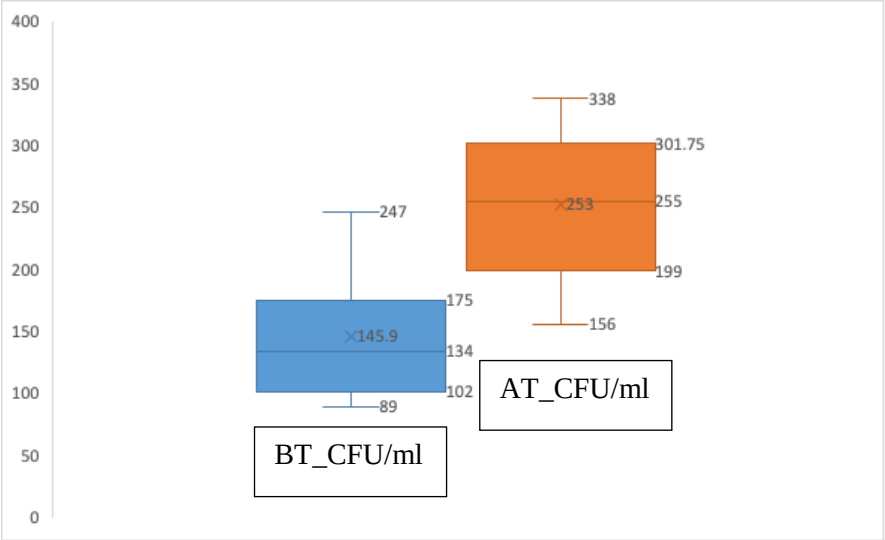
a) Effectiveness of Treatment on Visual Analogue Scale (VAS scale):



The box plot describes the before and after effect of intervention on VAS Scale. Lower and upper end of whisker represents the minimum and

maximum score. The mean VAS Scale before treatment was 5.6 which got reduced to 1.6.

b) Effectiveness of Treatment on Gut Microbiota:



The box plot describes the before and after effect of intervention on Gut Microbiota. The Mean CFU (colony forming units) before treatment was 145.9 and got increased to 253 after treatment.

Table No.3:- Bacterias which are present before treatment

BT	**No. of samples per patient**	**%**
Bifidobacteium longum	2	20
Lactobacillus acidophillus	4	40
Lactobacillus catenulatum	4	40
Total	10	100

Table No.4:- Bacterias which are present after treatment.

Bacterias present AT	**No. of samples per patient**	**%**
Bifidobacterium bifidum	1	10
Bifidobacteium longum	2	20
Lactobacillus acidophillus	4	40
Lactobacillus catenulatum	2	30
Total	10	100

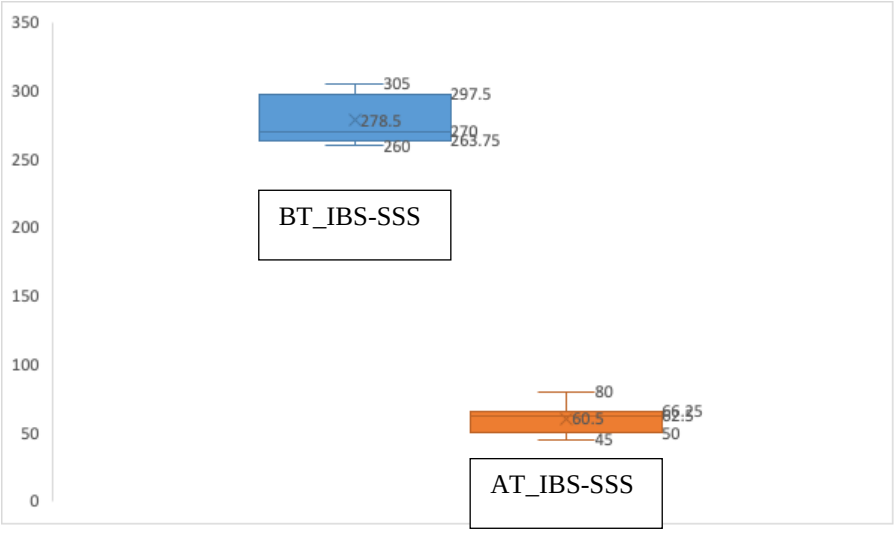
Figure 2: Before and treatment assessment

Figure 3: After treatment assessment:

c) Effectiveness of Treatment on Bristol stool form scale:

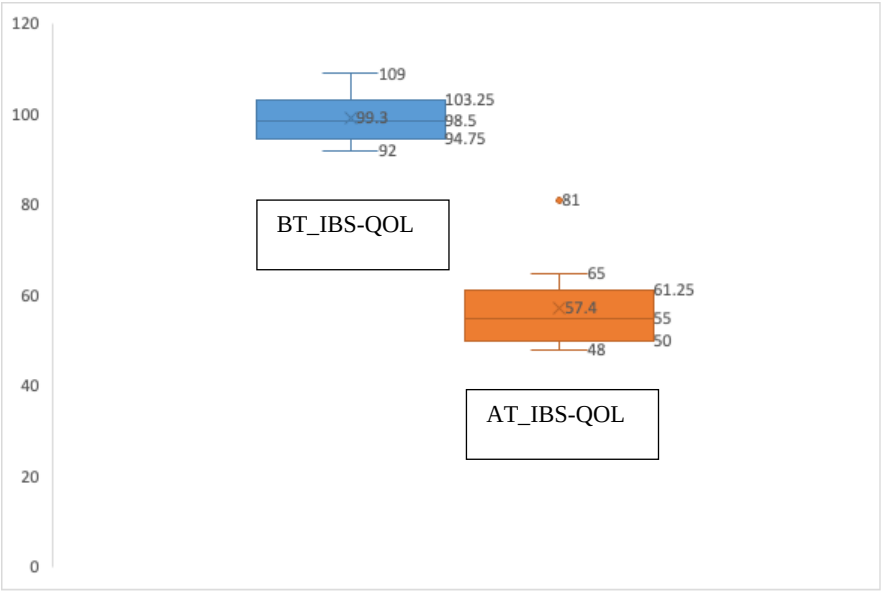
S.NO	BT		AT	
1	TYPE 2	TYPE 6	TYPE 3	TYPE 4
2	TYPE 1	TYPE 6	TYPE 3	TYPE 5
3	TYPE 2	TYPE 6	TYPE 2	TYPE 4
4	TYPE 2	TYPE 5	TYPE 3	TYPE 4
5	TYPE 2	TYPE 7	TYPE 3	TYPE 5
6	TYPE 2	TYPE 5	TYPE 3	TYPE 4
7	TYPE 2	TYPE 7	TYPE 3	TYPE 5
8	TYPE 1	TYPE 6	TYPE 3	TYPE 5
9	TYPE 2	TYPE 5	TYPE 3	TYPE 4
10	TYPE 2	TYPE 6	TYPE 3	TYPE 5

d) Effectiveness of Treatment on IBS- SSS:



The box plot describes the before and after effect of intervention on IBS-SSS. The mean IBS-SSS score before treatment was 278.5 and got reduced to 60.5 after treatment.

e) Effectiveness of Treatment on IBS-QOL:



The box plot describes the before and after effect of intervention on IBS-QOL. The mean IBS-QOL score before treatment was 99.3 and got reduced to 57.4 after treatment.

5. Discussion:

Irritable bowel syndrome (IBS) is a functional bowel disorder marked by recurrent abdominal pain that is related to defecation and associated with a change in stool form or frequency. IBS is not associated to structural or biochemical irregularities that are distinguishable with the present standard diagnostic techniques. Clinical diagnosis is established based on the signs and symptoms, and stool culture is used to investigate the biochemical parameters.Due to the multifaceted nature of the disease, the precise molecular pathophysiology is still poorly known, and the underlying pathogenesis is thought to be complex. Functional brain variations, altered intestinal motility, secretory dysfunctions, and somatic and psychiatric comorbidities are only a few of the functional changes that have been noted. Additionally, IBS has been linked to gastrointestinal disorders like immune activation, gut dysbiosis (microbial imbalance), reduced mucosal functioning, nerve sensitization, post-infectious plasticity, release of mucosal/ immunological mediators, and altered gene expression profiles.[11]

This clinical condition may be paralleled to*Grahaṇiroga* in Āyurveda, since one of *Grahaṇiroga's* cardinal characteristics is "The person voids stool regularly, either in solid or liquid form," citing *Caraka Ācārya* . Irritable Bowel Syndrome, generally can be linked to the above mentioned signs and symptoms.[12] The specific characteristics of Irritable Bowel Syndrome Mixed,

such as abdominal pain, passing stool slowly (constipated), and passing stool that is not fully formed (liquid consistency), can be interrelated.

Since *Mandāgni* is the underlying cause of both *Atisāra* and *Grahaṇiroga*, the general treatment protocol for *Grahaṇiroga* comprises the treatment given in *Ajīrṇa*and *Āmātisāra*conditions in accordance with ĀcāryaVāgbhaṭa. Controlling the disorganized*Agni* lessens the disease's symptoms and indications.[13]Treatment protocol containing drugs mentioned in the *VacāharidradiCūrṇa*, which have *Pācana* - *Dīpana* and *Āmātisārahara* qualities helps to restore the vitiated *Agni*.[14,15] The *Virecana Karma* with *Avipatti Cūrṇa*, as a whole result in *Indriya Prasādā, Koṣṭaśudi* and corrects the vitiated *Pitta Doṣa*.[16]*Priyaṅguambaśtādi*Cūrṇa, which has *Pakwatisārahara* action is administered as *Śamana Auṣadha*[17]along with *Takra*.*Takra* has similar to the action of probiotics over the intestinal flora and this regulates the dysbiosed gut microbiota.[18]

The effect of therapy and probable mode of action of *Grahaṇiroga*treatment protocol on various Assessment scales are detailed below:

- Effect of therapy on VAS scale:

Irritable bowel syndrome (IBS) is characterised by abdominal pain as a cardinal symptom; nevertheless, it is unclear how abdominal pain varies among IBS subtypes. Shah et al, conducted a survey study from 2020 used data from the National Gastrointestinal (GI) Survey to define the symptoms of abdominal pain among recognised IBS subgroups. The results point to parallels in how IBS-D and IBS-M exhibit abdominal discomfort, notably that both diseases are characterised by pain that is less uncomfortable, less frequent, and interferes with daily activities less than IBS-C. The suprapubic,

periumbilical, right, and left iliac regions were the most prevalent individual sites in all subtypes.[19]

All IBS subtypes are managed with antispasmodics (dicycylomine, otilonium, mebeverine) to alleviate symptoms such as abdominal pain and spasm. These medications are helpful for postprandial symptoms linked to an accentuated gastrocolonic reflex because they lower GI contractility.[20]The mechanism of action includes either anticholinergic or calcium channel blocking properties and the efficacy is superior to placebo for the prevention of recurrent IBS symptoms.[21]

Vacāharidrādhicūrṇa-Drugs like *Vacā*[22] (Acorus calamus), *Mustā*[23](Cyperusrotundus), *Devadāru*[24](Cedrus deodara), *Nāgara*[25](Zingiber officinale), *Abhayā*[26] (Terminaliachebula), *Haridrā*[27](Curcuma longa), *Yaṣṭi*[28](Glycyrrhizaglabra) and *Kuṭajā*[29] (Holarrhenaantidysentrica) exhibits spasmolytic effects and this explains its use in hyperactive states of gut like colic and diarrhea.

DāḍimādiGhṛta- Drugs like *Dāḍimā*[30] (Punicagranatum), *Dhānyaka*[31](Coriandrumsativum) and *Pippalī*[32](Piper longum) has spasmolytic action via voltage gated calcium channels.

PriyaṅguambaśtādiCūrṇa- Drugs like *Candana*[33](Santalum album) and *Bilva*[34] (Aeglemarmelos) has spasmolytic role in muscarinic receptors, 5-HT and calcium influx.

- Effect of therapy on Bristol stool form scale:

Most IBS-M patients go through cycles of reduced bowel movements per week, hard and interspersed with stools of varying consistency. In certain instances, this is the outcome of gradual stool accumulation during

constipation periods that leads to bowel purging. The therapeutic approach for these individuals is based on the same pharmacological choices as those mentioned for constipation and diarrhoea, and requires real-time modifications to meet the patient's symptoms. Chloride channel-related prostaglandin derivative that stimulates chloride secretion by acting on chloride channels on the intestinal enterocyte's apical membrane. This is followed by the passive entry of sodium ions and water into the lumen. Stools loosen and gastrointestinal transit quickens as a result.Antidiarrheals enhance segmental colonic contractions, lengthen the time it takes for contents to transit through the colon, raise anal pressure, and lessen rectal perception. By interacting with the GI muscles, these drugs prolong gastrointestinal transit time and facilitate more water absorption.[35]

Vacāharidrādicūrṇa- Drugs like *Mustā*[36](Cyperusrotundus),*Nāgara*[37](Zingiber officinale),*Ativiṣā*[38](Aconitum heterophyllum), *Abhayā*[39](Terminaliachebula), *Haridrā*[40](Curcuma longa) and *Kuṭajā*[29](Holarrhenaantidysentrica) possess action against the motility disorders by its Gut stimulatory and relaxant properties.

DāḍimādiGhṛta- Drugs like *Dāḍimā*[30](Punicagranatum), *Dhānyaka*[31](Corinadrumsativum), *Citrakā*[41](Plumbagozeylenica)and *Pippalī*[42](Piper longum) has antidiarrheal effect by its CCB activity.

PriyaṅguambaśtādiCūrṇa- Drugs like *Mañjiṣṭā*[43](Rubiacordifolia), *Samanga*[44](Mimisapudica), *Candana*[45](Santalum album),*Palāśa*[46](Buteamonosperma), *Kacchura*[47](Caempferiagalanga)and *Bilva*[48](Aeglemarmelos) shows antidiarrhoeal activity through inhibition of intestinal motility and antisecretory effects.

-Effect of Therapy on IBS-QoL questionnaire:

Antidepressants are recommended for IBS due to the co-existence of psychological problems, evidence that depression alters the central nervous system's reaction to painful stimuli, the advantages of antidepressants in prolonged painful conditions, and the need to rectify altered intestinal transit. Thus, SSRIs (selective serotonin re-uptake) shortens the orocecal transit time, whereas tricyclic antidepressants (TCAs) extend intestinal and oesophageal transit periods.Based on this, SSRIs are recommended in IBS with constipation(IBS-C) as the primary symptom, while TCAs are utilised in IBS-D. Antidepressants' multifaceted mode of action in IBS may involve decreased stimulation of pain centres in the anterior cingulate cortex and central pain processing, as well as peripheral pain-sensing systems including colonic compliance and visceral afferent function.[35]Consequence of an alteration in serotonin and norepinephrine is the cause of the high comorbidity between IBS and emotional distress, including anxiety and depressive symptoms. Neurotransmitters can be made by both the central nervous system and the enteric nervous system. Emotional distress may result from IBS disorders because of a decrease in the levels of serotonin and norepinephrine released and a decline in neurotransmitters' ability to bind to receptors.[49]

***Vacāharidrādhi Cūrṇa*-** Drugs like *Vacā*[50] (Acorus calamus), *Mustā*[51](Cyperusrotundus), *Devadāru*[52] (Cedrus deodara), *Nāgara*[53](Zingiber officinale), *Ativiṣā*[54](*Aconitum heterophyllum*), *Abhayā*[55](Terminaliachebula), *Haridrā*[56](Curcuma longa), *Yaṣṭi*[57](Glycyrrhizaglabra) and *Kalaśī*[58] (Desomdiumgangeticum) possess antidepressant activity by modulating the central neurochemical and HPA axis.

DāḍimādiGhṛta- Drugs like *Dāḍimā*[59](Punicagranatum), *Dhānyaka*[60] (Coriandrumsativum) and *Pippalī*[61](Piper longum)acts by increasing monoaminergic activity.

Priyaṅguambaśtādi Cūrṇa- Drugs like *Padma*[62] (Nelumbomucifera), *Ananta*[63](Tragiainvolucrata), *Mocarasa*[64] (Bombaxceiba), *Dhātaki*[65](Woodfordiafruiticosa), *Lodhra*[66](Symplococcusracemosa) and *Bilwa*[67] (Aeglemarmelos) exerts potent antidepressant like effects in behaviours involve the normalization of neurochemical abnormalities in the monoamine neurotransmitter system.

- Effect of therapy on Gut Microbiota Modulation:

A number of processes considered to be involved in the pathophysiology of IBS are mostly regulated by the gut microbiota. Commensals like Lactobacillus and Bifidobacterium may lessen the pathophysiology of IBS disease and ameliorate symptoms. IBS causes changes to the microbiota, and these changes may have an impact on the aetiology of the condition through, for example increased permeability, an altered immunological profile, impacts on the gut-brain axis, and modification of gut neuromuscular function. Patients with IBS were shown to have lower levels of lactobacilli and bifidobacteria, and their activities were severely hindered.[68]Additionally, there is proof that probiotics can normalise the interaction between pro- and anti-inflammatory cytokines via stabilising microbiota and affecting intestinal fermentation. Visceral sensitivity, intestinal permeability, and inflammation are all positively impacted by these findings. These commensals have been demonstrated to improve barrier function, prevent pathogen adherence to the gut lining, mitigate visceral

hypersensitivity, lessen overall IBS symptoms, induce the expression of m-opioid and cannabinoid receptors, control the gut-brain neuroendocrine axis, suppress the production of pro-inflammatory cytokines, and increase the expression of intestinal serotonin transporter when given as probiotics.Evidence from numerous research suggests that enteric pathogens like Shigella, Clostridium perfringens, Escherichia coli, Bacillus cereus, Salmonella, Pseudomonas aueroginosa, and Campylobacter species could pose a concern to people with dysbiosed state of IBS.[69,70]

Vacāharidrādicūrṇa- Drugs like *Vacā*[71] (Acorus calamus), *Devadāru*[72] (Cedrus deodara), *Nāgara*[73](Zingiber officinale), *Abhayā*[74](Terminaliachebula), *Haridrā*[75] (Curcuma longa), *Kalaśī*[76] (Desmodiumgangeticum) and *Kuṭaja*[77] (Holarrhenaantidysentrica) produces inhibition zone against S.aureus, Salmonella and E.coli.

DāḍimādiGhṛta- Drugs like *Dāḍimā*[78](Punicagranatum), *Dhānyaka*[79](Coriandrumsativum), *Citraka*[80](Plumbagozeylenica) and *Pippalī*[81](Piper longum) showed maximum antibacterial activity against gram negative (E.coli, Psuedomonas and S.typhi) and gram positive(S.aureus).

PriyaṅguambaśtādiCūrṇa- Drugs like *Puśpāñjana*[82](Zinc oxide), *Padma*[83] (Nelumbomucifera), *Yojanavalli*[84](Rubiacordifolia), *Anantā*[85](T. involucrata), *Mocarasa*[86](Bombaxceiba), *Samanga*[87](Mimosa pudica), *Punnāga*[88](Canophylluminophyllum), *Candana*[89] (Santalum album), *Dhātaki*[90](Woodfordiafruiticosa), *Ambaṣṭa*[91] (Spondiaspinnata), *Nandīvṛkṣa*[92](Tabernaemontana divaricate), *Palāśa*[93](Buteamonosperma), *Kacchura*[94](Caempferiagalanga), *Bilva*[95] (Aeglemarmelos) and *Katvanga*[96]

(Ailanthus excelsa) showed significant zone of inhibition against bacterial organisms like E. coli, S.aureus and P. aeruginosa.

Takra-Probiotics can maintain healthy intestinal function by balancing the microbiota in the intestines, which also prevent or treat a number of gastrointestinal illnesses such as infectious diarrhoea, antibiotic-related diarrhoea, and irritable bowel syndrome.In general, probiotic bacteria are LAB(Lactic acid bacteria) from the species Lactobacillus acidophilus, L. gasseri, L. helveticus, L. johnsonii, L. (para)casei, L. reuteri, L. plantarum, L. rhamnosus, and L. fermentum. However, Bifidobacterium species including B. bifidum and B. longum are also utilised. Numerous studies have demonstrated that buttermilk has a beneficial effect on commensal bacteria like Bifidobacterium and Lactobacillus.[97,98,99]

Discussion of Mode of Action on Treatment protocol:

IrritableBowel syndrome chiefly affecting the Gastro-intestinal system is identified as *Grahaṇiroga* in *Āyurveda*. This condition is understood as qualitative impairment in *Agni* (*Mandāgni*) due to the *Śārīrika* and *MānasīkaDoṣa Duṣṭi*. Hence, the treatment protocol necessitates improvement in functioning of the *Agni*. In *Āyurveda*, it is achieved through Elimination of the *KupitaDoṣas* to facilitate functioning of *Agni* and thereby preventing the occurrence of disease in the future.

1. ***Pācana - Dīpana with VacāharidradiCūrṇa***:

Agnimāndya is closely associated with *Grahaṇiroga* and as the subjects were found to have irregular appetite and improper digestion, initially *Pācana - Dīpana* was administered with *VacāharidradiCūrṇa* till *SamyakLaṅghana Lakṣaṇā*were observed. The *Virūkṣaṇa* property of

*Pācana*drugs (*Rūkṣa, Uṣṇa* and *LaghuGuṇa*) helps in the digestion of accumulated *Āma* in the *Koṣṭa*[100,101] whereas the *AgnibhūyiṣṭaGuṇa* of *Dīpana* drugs stimulates *Agni,* thereby preventing the further production of *Āma*.[102]*VacāharidradiCūrṇa* is *Kaṭu Tikta Rasa Pradhāna* and possess *UṣṇaVīrya, LaghuRūkṣaTīkṣṇaGuṇa* and have *Vāta Kapha Śamana* property. All the drugs have *Pācana and Dīpana* properties which effectively corrects the *Mandāgni* and promote proper digestion and metabolism.

2. ***Snehana and Swedana***:

Ābhyantara and *BāhyaSnehana* and *Swedana* altogether facilitates the proper mobilization of the *UtkliṣṭaDoṣas* by inducing the state of *Vṛddhī ViṣyandanaPāka*and *Vāyu Nigraha*.[103]

3. ***ĀbhyantaraSnehana with DāḍimadiGhṛta***:

After correcting the deranged *Agni* by *Pācana and Dīpana* , *Ābhyantara Snehana* is done by using *DāḍimadiGhṛta*. *Snehapāna* helps in *Utkleśana* [*DoṣaVṛddhī*]. It also helps in preventing further complications of the *Śodhana* procedure. Here in this condition, *Ghṛta*is most suitable for *Snehapāna* as it alleviates *Pitta* and *Vāta* and is *Agnikrut*. It is indicated for those desiring good strength (*Balā*), nourishment (*Puṣṭikāma*) and if it is administered judiciously lead to *Diptāntaragni*.[104] *DāḍimadiGhṛta*is a *TridoṣaŚamana Auṣadha Yoga* having action over the *Agni*. The *Ghṛta*has *Dīpana* property. *Ghṛta*when processed with drugs like *Śṛñgavera, Dāḍima, Pippalī*etc. by the *SaṃskārasyaAnuvartanāt* property, it attains the *Pācana and Dīpana* effect. Thus the *Ghṛta*increases the functioning capacity of

Agni as well as *Grahaṇi*. The *Kaṭu Rasa, Laghu, RūkṣaTīkṣṇaGuṇa* and *UṣṇaVīrya*of the drug, have dominance of *Agni, Ākāśa&VāyuMahābhūtās*. Moreover, *DāḍimadiGhṛta* brings about *MūḍaVātānulomana*. This also bestows to the improved functioning of *Agni*.[105]

4. ***Sarvānga Abhyanga and Bāshpa Sweda***:

Here, *BāhyaSnehana* is done with *Tila Taila* which possess *Sūkṣma, Tīkṣṇa*, *Uṣṇa, Vyavāyī* and *ViṣādaGunas* and is followed by *Bashpa Sweda* which possess *Uṣṇa, Tīkṣṇa, Sūkṣma* and *Sara Guna*. This is done as a *Pūrvakarma* to *Śōdhana*.[106,107]

5. ***KoṣṭaŚōdhana***:

The *Sneha* bound *UtkliṣṭaDoṣas* which are in the gut are eliminated out of the body through *ŚōdhanaKarmas*. Here, *Avipatti Cūrṇa* is administered for *Koṣṭa Śōdhana* as it is considered as the drug of choice for *Virechana* in patients having *Paittika* diseases, *Agnivivikārās*, also it aids in correcting *Agni* and is *Viṣahara* in nature.[16]

6. ***ŚamanaAuṣadha with PriyaṅguambaśtādiCūrṇa***:

PriyaṅguambaśtādiCūrṇa is a poly-herbal combination of 19 drugs which is mainly indicated in *PakvaAtisāra* and is *VraṇaRopaṇa* and *Sandhānīya*and *Pittahara*in nature. Drugs like *Priyaṅgu*, *Bilwa*, *Katvanga* which are *Dīpana* helps in maintaining the quality of *Agni* attained through the process of *Virecana*. Drugs like *Priyaṅgu, Padma, Mānadruma, Samanga, Punnāga, Dhātaki,Madhūka*, *Namaskāri*, *Nandivṛkṣa*, *Lodhra* are *Grāhi* and aid in *PurīṣaSangrahana*.[17]

7. ***Takra as Anupāna***:

Takra is predominant of *AmlaRasa* and Kaṣāya*Anurasa*. It does *Tridoṣahara, Agni Dīpana, Hṛdhya, Kapha Vāta*and acts as *Grāhi*.Due to *Kaṣāya Anurasa*, *UṣṇaVīrya&Rūkṣa*and*Vikāśi*, it brings down aggravated *Kapha*. Due to its *Madhura, AmlaRasa, SāndraGuna*, helps to correct the vitiated *SamānaVāyu*. Due to its *Madhura Vipāka* helped in the balance of *Pitta*.[18] Hence *Takra* as *Anupāna* to *Priyaṅguambaśtādi*Cūrṇa adds its *Pakwa Atisāra* activity and aid in *Śamana*of *GrahaṇiDoṣa*.

6. Conclusion:

Thus, *Grahaṇiroga* Treatment Protocol helped in Gut Microbiota modulation and regulation by significant changes in IBS-SSS, IBS-QoL, VAS Scale, BSF Scale (Gut Microbiota Assessment). The effect of treatment protocol shows statistical significance of p value <0.05 in the assessment scales.The pharmacological action of *Grahaṇi* treatment protocol mainly as *Pācana - Dīpana*, *Amātisara*, *Pittahara*, and *PakwaAtisāra*actions in it. These helps in regulating the abdominal pain, altered gastrointestinal motility, quality of life and dysbiosed gut of the patient.

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