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Beneficial Effect of the Combination of Allopathic, Ayurvedic and Probiotic Treatment in Acetic Acid-Induced Ulcerative Colitis in Rats

Author: R. Padmavathi[1]

Co Authors: Nousheen Fathima[2] and Mohammed Abdul Samad[3]

[1-3]Department of Pharmacology, G. Pulla Reddy College of Pharmacy, Osmania University, Hyderabad, Telangana, India

ABSTRACT

The goal of the current research was to explore the beneficial effect of integrating allopathic, *ayurvedic*, and probiotic treatment in acetic acid-induced ulcerative colitis in rats. Wistar rats of either sex were divided into six groups. Group I received a single dose of vehicle rectally, group II received 2 ml of 3% v/v of acetic acid in 0.9% saline rectally, group III received mesalazine (100 mg/kg/p.o), group IV received *Grahi* (10 mg/kg/p.o), group V received mesalazine and *Grahi* and group VI received mesalazine, *Grahi* and sprolac (100 mg/kg/p.o). The Disease Activity Index (DAI) was measured during the treatment period. Blood was withdrawn white blood cell and differential leucocyte count at the conclusion of the research. The animals were sacrificed at the end of study period for the evaluation of intestinal inflammation, colonic microbial count, myeloperoxidase concentrations and histopathology under ether anesthesia. Combination treatment displayed a major beneficial impact relative to monotherapy in relieving colitis. Especially in comparison to monotherapy, total improvements in DAI, inflammatory ratings, white blood cells count, colonic microbial count and myeloperoxidase indicated a substantial improvement, reflecting the preference for combined therapy. Although monotherapy has also been successful in improving colitis, combined therapy has been even better. In colitis, the application of mesalazine and *Grahi* may be more helpful than monotherapy. In order to produce the most effective result in the treatment of colitis and other inflammatory intestinal disorders, probiotics may be prescribed as an adjuvant to the mixture of mesalazine and *Grahi*.

Key Words *Mesalazine, Grahi, Sprolac, Acetic Acid, Colitis, Disease Activity Index*

INTRODUCTION

Inflammatory Bowel Syndrome (IBS) involves two main gastrointestinal tract idiopathic inflammatory disorders. They are Ulcerative Colitis (UC) and Crohn's Disease (UC). These are also categorized by recurring acute inflammatory episodes and recovery cycles that interrupt the lifestyle of a patient[1]. Ulcerative colitis is defined by mucosal inflammation which is continually confined to the colon, rectum, which extends proximally and also affects the area of the periappendicea. On the other hand, Crohn's disease, characterized by transmural non-caseating granulomatous inflammation in any portion of the GIT, most usually progresses non-

continuously to the terminal ileum or perianal area[2,3].

While major complex inflammation is attributed to dysfunction of immune function to intestinal flora and activation of T-cells in genetically predisposed individuals, the current cause of UC is unclear. Based on the region surveyed, the estimated occurrence of UC varies from 1 to 10 cases per 100,000 persons. It is most prevalent in developing regions, particularly in western countries. Incidentally, in developed countries following western lifestyles, an increased prevalence of IBS has been reported. The total projected IBS population in India (2010) was 1.4 million, the second largest number after the US, with 1.64 million[4].

Mesalazine (5-amino salicylic acid) is a systemic anti-inflammatory drug that works by reducing inflammation induced by mucosal development of arachidonic acid compounds by cyclooxygenase and lipoxygenase processes and by inhibiting the activation of nuclear factor kappa B, thus reducing the production of peroxisome proliferative activated by the production of pro-inflammatory cytokines[5]. *Grahi* is an *ayurvedic* formulation which is already in clinical use, the components of which are *Punica granatum* (anti-inflammatory, antioxidant), *Woodfordia fructicosa* (anti-inflammatory, analgesic), *Ajmoda* (anti-inflammatory, antibacterial, gastroprotective), *Piper longum* (antioxidant, immunomodulatory), *Zingiber Officinalis* (anti-inflammatory, immunomodulatory)[6]. Probiotics *Lactobacillus acidophilus* strain (Sporlac) has been selected which is used for intestinal infections, diarrhoea, abnormal intestinal fermentation, irregular bowel movement, constipation, dyspepsia, flatulence, impaired digestion, and absorption. It comprises 150 million Lactobacillus strains per gram[7].

Therefore, the current investigation was conducted to explore the beneficial impact of the mixture of allopathic, *ayurvedic* and probiotic treatment in acetic acid-induced ulcerative colitis in rats on the basis of the distinct anti-inflammatory and gastroprotective properties of the afore mentioned medicines.

MATERIALS AND METHODS

Drugs and reagents

Mulletin broth was purchased from Hi-Media, India, Leishman's strain was purchased from NICE-Chemicals Pvt Ltd., Agar Agar and WBC fluid were purchased from S.D Fine Chemicals Ltd., Hexadecyltrimethyl Ammonium Bromide (HTAB) and Orthodianisidine Hydrochloride were purchased from Sigma Aldrich, Mesalazine, Sprolac, and other rectal catheters were purchased from Mawa medical and surgical, *Grahi* and acetic acid were purchased from standard sources.

Experimental animals

Wistar rats of either sex (150-200 g) were obtained from Sainath Agency, Hyderabad, India. They were sustained within normal operating conditions and had unrestricted access to standard rat feed and water ad libitum (12-h light/dark period, 25 ± 3 °C, and 45-65 percent humidity). For a week prior to the start of the trial, all animals were

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acclimatized to laboratory environments. All the experimental activities were carried out in accordance with the guidelines of the committee for the management and regulation of animal experimentation (CPCSEA). The institutional animal ethics committee (IAEC) of G. Pulla Reddy College of Pharmacy, Hyderabad, India has reviewed and accepted the report (GPRCP/IAEC/20/16/02/PCL/AE-2-RATS-M-36).

Induction of ulcerative colitis in rats

In overnight fasting rats, colitis was induced by intra-rectal administration of acetic acid through ether anaesthesia. Nelaton catheters with a diameter of 2 mm were implanted into the colon at a distance of 8 cm via the rectum. A 2 ml solution of 3% v/v acetic acid in 0.9% saline was instilled into the colon lumen and held for 30s in the supine trendelenburg location to avoid the intracolonic instillate from leaking (**Figure 1**)[5].

Experimental design

Thirty-six wistar rats were broadly assigned into six groups of 6 rats each. Colitis, as seen in **Table 1**, was caused in all the experimental groups except sham control.

Table 1 Animal Grouping

Groups	Treatment
I	0.5% CMC in normal saline
II	2 ml of 3% v/v of acetic acid in 0.9% saline at a single dose
III	Mesalazine (100 mg/kg/p.o) for 7 days
IV	*Grahi* (10 mg/kg/p.o) for 7 days
V	Mesalazine (100 mg/kg/p.o) & *Grahi* (10 mg/kg/ p.o) for 7
VI	Mesalazine (100 mg/kg/p.0), *Grahi* (10 mg/kg/p.0) & Sporlac (100 mg/kg/p.o) for 7 days)

The Disease Activity Index (DAI) was measured during the treatment period. Blood was withdrawn by a retro-orbital puncture for WBC and DLC count at the conclusion of the research. The animals were then sacrificed under ether anesthesia and colon was dissected out, flushed gently with saline, and used for macroscopic scoring, colonic bacterial count, myeloperoxidase estimation, and histopathology.

Evaluation of Disease Activity Index (DAI)

Colitis was measured on a regular basis with a DAI measuring weight loss, stool quality and rectal bleeding for 7 days in all groups during the treatment time. Scoring was given as shown in **Table 2**[8].

Table 2 Scoring for DAI

Score/points	Weight loss	Stool consistency	Rectal bleeding
0	No Weight Loss	Well-formed pellets	No blood in hemoccult
1	1 to 5%	-	-
2	5 to 10%	Pasty and semi-formed stools that did not stick to the anus	Positive hemoccult
3	10 to 20%	-	-
4	> 20%	Liquid stools that did stick to the anus	Gross bleeding

White Blood Cells (WBC) count and Differential Leucocyte Count (DLC)

Blood was obtained into EDTA coated appendron tubes by retro-orbital puncture. After 1:10 blood

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dilution in WBC diluting solution, cumulative WBC counts were done on a hemocytometer. With Leishman's stain, DLC was performed. Smears were analysed at 100x magnification and, depending upon nuclear morphology and cytoplasmic staining properties, the percentage of lymphocytes, monocytes, eosinophils, basophils and neutrophils was performed[9].

Figure 1 Induction of Colitis in Rat

Evaluation of macroscopic characters

The macroscopic scoring system for each animal assessed the severity of colitis. To extract faecal traces, the distal part (2 cm) of the colon was removed and longitudinally sliced and washed in physiological saline. Using the following score pattern, macroscopic inflammation scores were given depending upon the clinical characteristics of the colon. Scoring were done as 0 in case of no macroscopic changes; 1 in case of mucosal erythema alone; 2 in case of mild mucosal oedema, minimal bleeding or minor erosion; 3 in case of significant oedema, bleeding ulcer or erosion; and 4 in case of extreme ulceration/erosion, oedema and tissue necrosis[10].

Colonic bacterial count

The mucosal sample was collected by scratching the mucosa with sterile cotton bud, and immediately transferred into 2% of sterile nutrient broth & incubated for 24 hrs at 37°C aerobically. From this, 10 µl of the sample was withdrawn and plated on Mulletein agar medium and was incubated at 37°C for 24hrs. The Colony Forming Units (CFU) was calculated[11,12].

Assessment of Colonic Myeloperoxidase (MPO) Activity

Inflamed colon fragments were removed and washed with ice-cold saline, cleaned, measured and excised. The tissue was homogenized with tissue homogenizers in 10 volumes of ice-cold potassium phosphate buffer (pH: 7.4). The homogenate was centrifuged at 3500 rpm for 30 min at 4°C. The supernatant was discarded; 10ml of ice-cold 50 mM potassium phosphate buffer (pH: 6.0) containing 0.5% Hexadecyltrimethyl ammonium bromide (HETAB) and l0 mM EDTA was then added to the pellet. It was then subjected to one cycle of freezing and thawing for a brief period (l5 s) of sonication. After sonication, solution was centrifuged at 15,000 rpm for 20 min. With 2.9 ml of 50mM phosphate buffer containing 0.167 mg/ml O-dianisidine hydrochloride and 0.0005 percent H_2O_2, 0.1 ml of supernatant was mixed. The absorption spectrum shift was spectrophotometrically calculated at 460 nm[13,14].

MPO activity = X / wt of the tissue piece taken

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Where, X = 10* change in absorbance per minute / Volume of supernatant taken in the final mean.

Histopathological study

In 10% of formalin, trapped in paraffin, colonic specimens were immersed and sliced into 5µm pieces. For the measurement of tissue damage, paraffin sections were treated with xylene, hydrated and stained with Haematoxylin and Eosin (H&E)[15].

Statistical analysis

Results were expressed as Mean ± S.E.M. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test in Graph Pad Prism 7.0 software. $P < 0.05$ was considered significant.

RESULTS

Effect on Disease Activity Index (DAI)

Treatment with either mesalazine (100 mg/kg) or *Grahi* (10 mg/kg) alone showed the moderate incidence of weight loss, diarrhoea and rectal bleeding compared to colitis control group. The lowest incidence of weight loss, diarrhoea and rectal bleeding was observed with the combination of mesalazine & *Grahi* compared to monotherapies. However, no significant difference was observed by the addition of probiotic to mesalazine and *Grahi* (**Figure 2**).

Effect on leucocyte count

Induction of colitis significantly ($P < 0.0001$) increased WBC count in colitis control group compared to sham control group. Treatment with

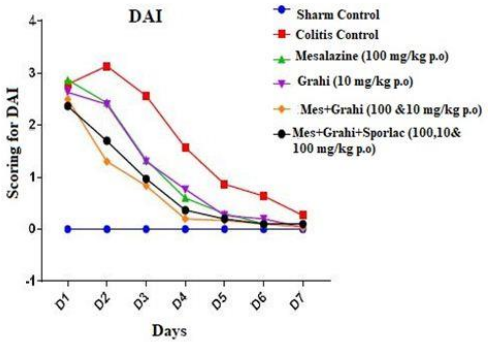

Figure 2 Effect of Mesalazine, *Grahi* & Sporlac on DAI in Acetic Acid Induced Colitis in Rats
Results are expressed as mean ± S.E.M, $^{a}P < 0.0001$ vs. sham control. $^{\alpha}P < 0.0001$,$^{\beta}P < 0.001$, $^{\gamma}P < 0.01$ vs. Colitis control $^{\#}P < 0.0001$, $^{\exists}P < 0.01$ vs. Mesalazine, $^{\%}P < 0.01$,$^{*}P < 0.05$ vs. *Grahi*.

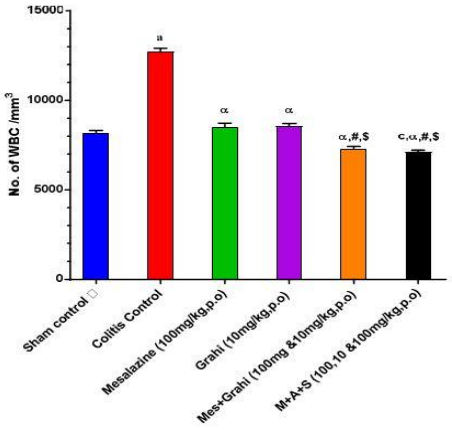

Figure 3 Effect of Mesalazine, *Grahi* & Sporlac on WBC Count in Acetic Acid Induced Colitis in Rats
Results are expressed as mean ± S.E.M, $^{a}P < 0.0001$, $^{c}P < 0.01$, vs Sham control, $^{\alpha}P < 0.0001$, vs. colitis Control, $^{\#}P < 0.0001$, vs Mesalazine, $^{\$}P < 0.0001$, vs *Grahi*.

either mesalazine (100 mg/kg) or *Grahi* (10 mg/kg) alone has shown a significant ($P < 0.0001$) decrease in the WBC count compared to colitis control group. Treatment with a combination of mesalazine & *Grahi* has also shown significant ($P < 0.0001$) decrease in the WBC count compared to monotherapies. However, no significant ($P <$

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0.01) difference was observed by the addition of probiotic to mesalazine & *Grahi* (**Figure 3**).

In DLC, treatment with either mesalazine (100 mg/kg) or *Grahi* (10 mg/kg) alone significantly ($P < 0.0001$ & $P < 0.01$) decreased the neutrophil count compared with colitis control group. Treatment with a combination of mesalazine & *Grahi* has shown a significant ($P < 0.0001$ & $P < 0.01$) decrease in the neutrophil count compared to the monotherapies. However, no significant ($P < 0.01$) difference was observed by the addition of probiotic to mesalazine & *Grahi* (**Figure 4**). No difference has been observed in eosinophils, basophils, lymphocytes and monocytes between experimental groups.

Effect on macroscopic characters

Treatment with either mesalazine (100 mg/kg) or *Grahi* (10 mg/kg) alone significantly ($P < 0.0001$ & $P < 0.01$) reduced the macroscopic score compared to colitis control group. Treatment with a combination of mesalazine & *Grahi* has shown a significant ($P < 0.0001$) reduction in the macroscopic score compared to monotherapies. However, no significant ($P < 0.05$) difference was observed by the addition of probiotic to mesalazine & *Grahi* (**Figure 5 & 6**).

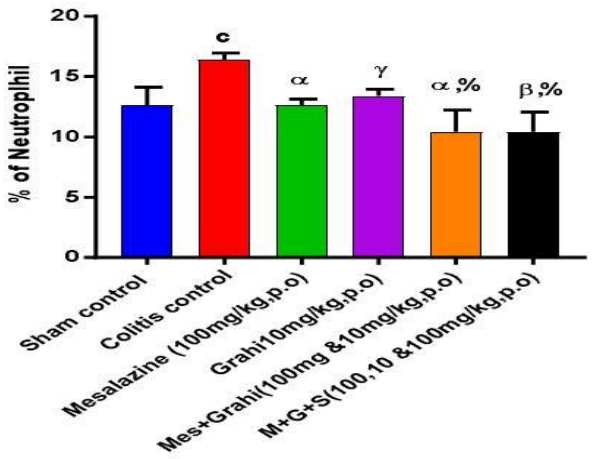

Figure 4 Effect of Mesalazine, *Grahi* & Sporlac on Neutrophil Count in Acetic Acid Induced Colitis in Rats

Results are expressed as mean ± S.E.M, $^{c}P < 0.001$ vs. sham control. $^{\alpha}P < 0.0001$, $^{\beta}P < 0.001$, $^{\gamma}P < 0.01$ vs. Colitis control, $^{\%}P < 0.01$, vs. *Grahi*.

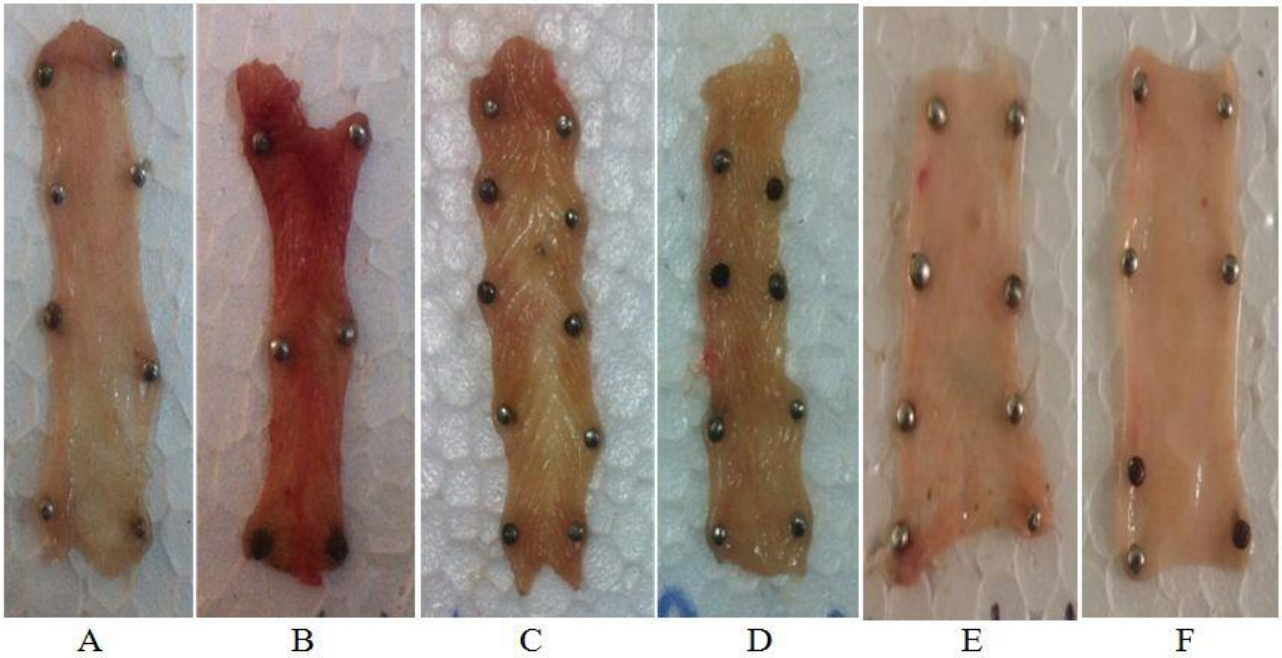

Figure 5 Macroscopic Scoring for Colon

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Macroscopic presentation of acetic acid induced ulcerative colitis in rats. A: Sham control; B: Colitis colon treated only with acetic acid rectally; C: UC treated with mesalazine (100 mg/kg); D: UC treated with *Grahi* (10 mg/kg); E: Treated with mesalazine & *Grahi*; F: Treated with mesalazine, *Grahi* & sporlac (100 mg/kg/).

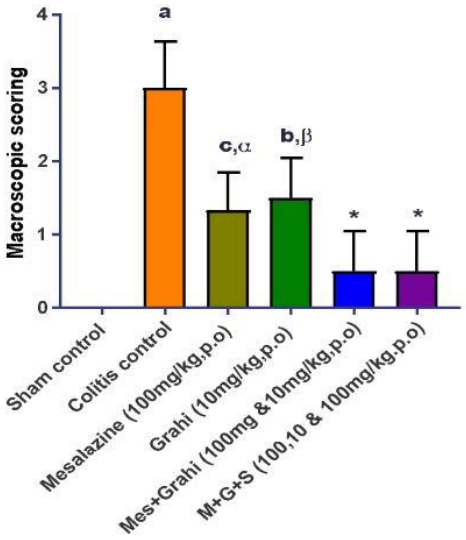

Figure 6 Effect of Mesalazine, *Grahi* & Sporlac on Macroscopic Score in Acetic Acid Induced Colitis in Rats

Results are expressed as mean ± S.E.M, $^{a}P < 0.0001$, $^{b}P < 0.001$, $^{c}P < 0.01$ vs sham control, $^{\alpha}P < 0.0001$, $^{\beta}P < 0.001$ vs colitis control, $^{*}P < 0,05$ vs *Grahi*.

Colonic bacterial count:

Treatment with either mesalazine (100 mg/kg) or *Grahi* (10 mg/kg) has shown significant ($P < 0.0001$ & $P < 0.001$) increased in microbial count compared to colitis control group. Treatment with a combination of mesalazine & *Grahi* has shown a significant ($P < 0.0001$) increase in the colonic microbial count compared to monotherapies. Addition of probiotic to combination of mesalazine & *Grahi* has also shown more significant ($P < 0.0001$) increase in the colonic microbial count (**Figure 7 & 8**).

Assessment of Colonic Myeloperoxidase (MPO) activity

Treatment with either mesalazine (100 mg/kg) or *Grahi* (10 mg/kg) alone significantly ($P < 0.0001$ & $P < 0.001$) decreased the MPO levels compared to colitis control group. Treatment with combination of mesalazine & *Grahi* has shown a significant ($P < 0.0001$) decrease in the MPO levels compared to monotherapies. Also, a significant ($P < 0.0001$) difference was observed by the addition of probiotic to mesalazine & *Grahi* combination (**Figure 9**).

Histopathological study

Sham control group showed normal architecture with the muscularis mucosa, the sub mucosa and the mucosa with the normal epithelial lining of the crypts, the goblet cells and the lamina propria (**Figure 10A**). Colitis control animal showed the disruption of the epithelial mucous layer of the villi with the necrotic material in the lumen (**Figure 10B**). Mesalazine (100 mg/kg/p.o), *Grahi* (10 mg/kg/) attenuated the extent and severity of the histological signs of cell damage (**Figure 10C & D**). Mesalazine, *Grahi* & sporlac (100 mg/kg) revealed intact epithelial lining with normal muscularis mucosa, submucosa and mucosa (**Figure 10E & F**).

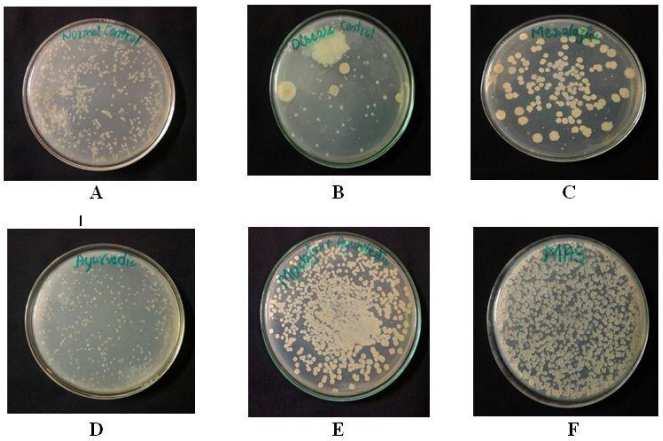

Figure 7 Colonic Microbial Count

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Colonic microbial count of acetic acid induced ulcerative colitis in rats. A: Sham control; B: Colitis colon treated only with acetic acid rectally; C: UC treated with mesalazine (100mg/kg); D: UC treated with *Grahi* (10 mg/kg); E: Treated with mesalazine & *Grahi*; F: Treated with mesalazine, *Grahi* & sporlac (100 mg/kg/).

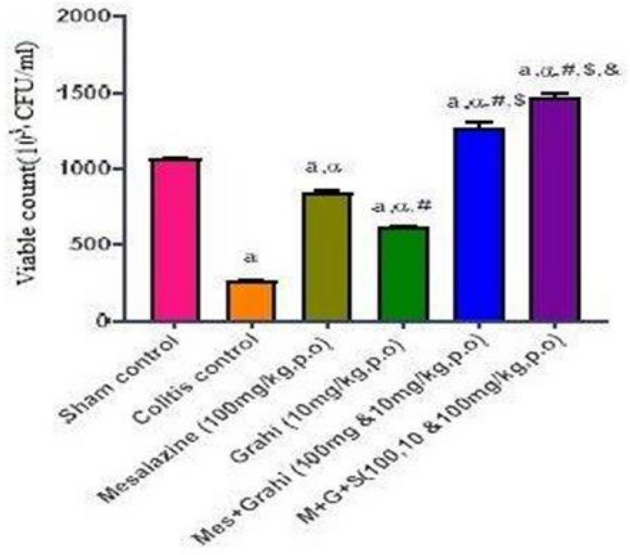

Figure 8 Effect of Mesalazine, *Grahi* & Sporlac on Colonic Microbial Count in Acetic Acid Induced Colitis in Rats

Results are expressed as mean ± S.E.M, $^{a}P < 0.0001$ vs Sham control, $^{\alpha}P < 0.0001$ vs Colitis control, $^{\#}P < 0.0001$ vs Mesalazine, $^{\$}P < 0.0001$ vs *Grahi*, $^{\&}P < 0.0001$ vs Mesalazine & *Grahi*.

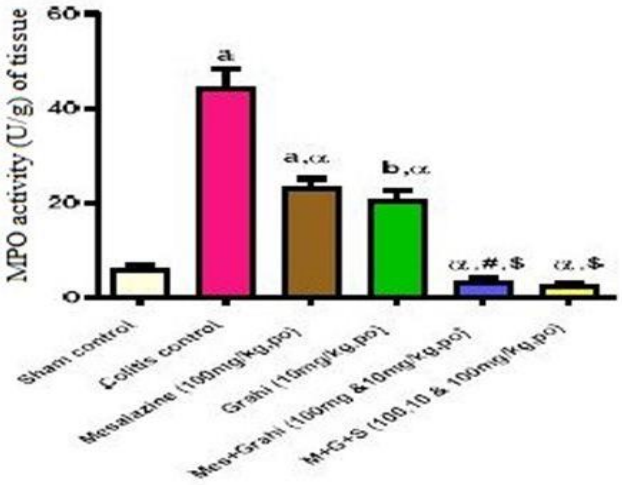

Figure 9 Effect of Mesalazine, *Grahi* & Sporlac on MPO Levels in Acetic Acid Induced Colitis in Rats

Results are expressed as mean ± S.E.M, $^{a}P<0.0001$, $^{b}P<0.001$vs Sham Control, $^{\alpha}P <0.0001$ vs. colitis control, $^{\#}P<0.0001$ vs Mesalazine, $\$P<0.0001$ vs *Grahi*.

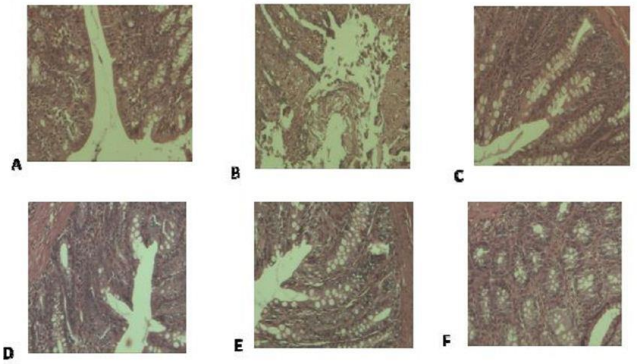

Figure 10 Showing 5µm Thick Haematoxylin & Eosin Stained Section of the Rat Colonic Tissue under 10 x Magnifications

A: Sham control; B: Colitis colon treated only with acetic acid rectally; C: UC treated with mesalazine (100mg/kg); D: UC treated with *Grahi* (10 mg/kg); E: Treated with mesalazine & *Grahi*; F: Treated with mesalazine, *Grahi* & sporlac (100 mg/kg/).

DISCUSSION

Ulcerative colitis is a persistent gastrointestinal tract inflammatory bowel condition marked by excessive diarrhoea, stomach pain, nausea, anemia, weight loss and chronic recurring bowel ulceration. Pathophysiology of UC involves multiple mechanisms such as chronic immune system activation which results in continued infiltration of polymorphonuclear and mononuclear cells and discharge of inflammatory mediators such as cytokines, chemokines, and eicosanoids[16].

The current treatment options for UC are allopathic and some *ayurvedic* drugs. But due to increasing complications of UC, no single therapy is effective in completely curing and maintaining safety for long period. Studies have now switched to targeting combination therapy over monotherapy. Several researchers have found that combined therapy is much more beneficial than monotherapy[17].

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Moeinian et al.,[18] have proved the priority of combination of butyrate, *Lactobacillus casei* and *Lactobacillus carnitine* in ameliorating the severity of colitis in comparison to monotherapies Fatima & Shaza[19] have proved the combination therapy with Sulfasalazine plus Olmesartan was more effective than single drug treatment. As the combination therapy has proved more beneficial than monotherapies, the current research work was planned to investigate the beneficial effect of the combination of mesalazine (anti-inflammatory), *grahi* (immunomodulatory) and probiotic over monotherapies.

One of the standard models of UC is the induction of colitis by acetic acid in rats. Acetic acid causes epithelial necrosis, edema of the mucosa, sub-mucosa and also causes generation of reactive oxygen metabolites along with infiltration of activated neutrophils. The similarity to acute human intestinal inflammation is indicated by the mechanism involved in this model. The present study used acetic acid-induced colitis as an experimental model. Colitis was induced by inserting a neleton catheter to a distance of 8cm in the rectum under ether anesthesia. A solution of 2ml of (3%v/v) acetic acid in 0.9% saline was instilled into the lumen of the colon and maintained in inverted position for 30 sec[20]. The effectiveness of therapies was investigated by measuring DAI, WBC count, DLC count, macroscopic score, colonic microbial count, MPO and histopathology.

As UC is characterized by severe weight loss, bloody stools and frequent diarrhoea, DAI was used as the measure to evaluate these symptoms, which gives scoring based on the severity of the symptoms. The highest rate of weight loss, diarrhoea, and rectal bleeding was observed in the colitis control group. The treatment with either mesalazine or *Grahi* alone showed the moderate incidence whereas lowest incidence of weight loss, diarrhoea and rectal bleeding was observed with the combination of mesalazine & *Grahi* when compared to the monotherapies. But, no difference was observed by the addition of probiotic to mesalazine and *Grahi* combination.

UC involves activation of immune system and migration of activated neutrophils. So, the immunomodulatory role of combination therapy was investigated by evaluating WBC count & DLC count[16]. Induction of colitis significantly increased WBC count in colitis control group showing evidence of inflammation. Treatment with either mesalazine or *Grahi* alone has decreased the WBC count whereas combination of both has shown a more significant decrease in the WBC count when compared to monotherapies. The addition of probiotic has not shown any beneficial effects. Also, no difference has been observed in eosinophils, basophils, lymphocytes, and monocytes between experimental groups. However, induction of colitis significantly increased the neutrophil count in colitis control group which was decreased upon treatment with either mesalazine or *Grahi* alone and in combination. But, addition of probiotic to combination therapy doesn't show additive effects.

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The severity of ulcer was evaluated by the macroscopic scoring system. Induction of colitis has significantly increased the ulcer score and the macroscopic score in colitis control group compared to sham control group. This ulcer score was lowered by the treatment with either mesalazine or *Grahi* alone and combination. The probiotics combination here also does not show any additive beneficial effects.

In UC patients, the distribution of the enteric bacteria is changed, leading to dysbiosis, where the number of pathogenic bacteria is increased and the number of beneficial bacteria is decreased[21]. Induction of colitis has significantly decreased the colonic microbial count in colitis control group compared to the sham control group which was enhanced by the treatment either with mesalazine or *Grahi* and a combination of both. Addition of probiotic to combination has shown more increase in the colonic microbial count.

In order to kill bacteria, MPO, a heme-enzyme, is released drastically from neutrophils. It facilitates the production of cytotoxic agents like hypochlorite acid (HOCL), the stimulation of Nuclear factor-κB (NF- κB), which is able to induce other inflammatory factors *via* H_20_2[22]. Induction of colitis significantly increased the colonic MPO level in colitis control group which was decreased upon treatment with either mesalazine or *Grahi* alone and combination. To our revelation, a significant difference was also observed by the addition of probiotic to the combination therapy.

Histological findings such as mucosal edema, disruption of the epithelial mucosal layer of the villi and necrosis of the colon are considered a reliable sensitive indicator of the severity and extent of the inflammatory response[18,19]. Animals treated with acetic acid alone depicted the disruption of the epithelial mucous layer of the villi with the necrotic material in the lumen. The disrupt architecture of colon was retained normal upon treatment with mesalazine, *Grahi* and sporlac alone and in combination which prevented the severity of cell damage. Combination therapy has significantly attenuated colitis induced histopathological alterations compared to monotherapies.

CONCLUSION

Addition of probiotic to combination of mesalazine and *Grahi* has not shown any significant effect in attenuating DAI, ulcer index, WBC count, neutrophil count and MPO levels. However, there was substantial increase in the colonic microbial count with the addition of probiotic to mesalazine and *Grahi*. From the results obtained in the present study, it was concluded that combination of mesalazine (100 mg/kg) and *Grahi* (10 mg/kg) (allopathic and *ayurvedic*) could be more beneficial in ulcerative colitis than monotherapies. To produce a more favourable effect on ulcerative colitis, probiotics may be prescribed as an adjuvant to the mixture of mesalazine and *Grahi*. The current study needs to

be tested in more clinical trials for application in humans.

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REFERENCES

1. McDowell, C., Farooq, U., & Haseeb, M. (2020). Inflammatory Bowel Disease. [Updated 2020 Jun 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470312/

2. Lee, J. M., & Lee KM. (2016). Endoscopic Diagnosis and Differentiation of Inflammatory Bowel Disease. Clinical Endoscopy, 49(4), 370-5.

3. Larsen, S., Bendtzen, K., & Nielsen, O. H. (2010). Extraintestinal manifestations of inflammatory bowel disease: Epidemiology, diagnosis, and management. Annals of Medicine, 42, 97–114.

4. Kedia, S., & Ahuja, V. (2017). Epidemiology of Inflammatory Bowel Disease in India: The Great Shift East. Inflammatory Intestinal Diseases, 2, 102–15.

5. Bantel, H., Berg, C., Vieth, M., Stolte, M., Kruis, W., & Schulze-Osthoff, K. (2000). Mesalazine inhibits activation of transcription factor NF-kappaB in inflamed mucosa of patients with ulcerative colitis. American Journal of Gastroenterology, 95(12), 3452-7.

6. Daneshwari, K., & Kamal, N. D. (2020). A Critical Review on Stambhana and *Grahi* Karma: Concept Based Treatment Modalities. International Journal of Ayurveda and Pharma Research, 41–8.

7. Gareau, M. G., Sherman, P. M., & Walker, W. A. (2010). Probiotics and the gut microbiota in intestinal health and disease. Nature Reviews Gastroenterology & Hepatology, 7(9), 503-14.

8. Albert, E. J., Duplisea, J., Dawicki, W., Haidl, I. D., & Marshall, J. S. (2011). Tissue eosinophilia in a mouse model of colitis is highly dependent on TLR2 and independent of mast cells. American Journal of Pathology, 178(1), 150-60.

9. Samad, M. A., Padmavathi, R., Dixith, A. S., & Shirisha, K. (2020). Ameliorative Potential of Sap of *Borassus flabellifer* Against Cyclophosphamide Induced Myelotoxicity In Wistar Rats. British Journal of Pharmaceutical Research, 5(1), 2245-2253.

10. Paine, E. R. (2014). Colonoscopic evaluation in ulcerative colitis. Gastroenterological Report (Oxf), 2(3), 161-8.

11. Montelius, C., Osman, N., Weström, B., Ahrné, S., Molin, G., Albertsson, P. Å., & Erlanson-Albertsson, C. (2013). Feeding spinach thylakoids to rats modulates the gut microbiota, decreases food intake and affects the insulin response. Journal of Nutritional Science, 2, e20.

12. Punja, Z. K., Collyer, D., Scott, C., Lung, S., Holmes, J., & Sutton, D. (2019). Pathogens and Molds Affecting Production and Quality of *Cannabis sativa* L. Frontiers in Plant Science, 10, 1120.

13. Ahl, D. (2010). MPO measurements in DSS-treated mice. Uppsala University, 5-18.

14. Patil, N. R., Rasal, V. P., & Malabade, R. H. (2014). Screening of Mandarin Oil on Indomethcin Induced Inflammatory Bowel Disease in Wistar Rats. Indian Journal of Pharmaceutical Education and Research, 48, 1–6.

ORIGINAL RESEARCH ARTICLE

15. Taqi, S. A., Sami, S. A., Sami, L. B., & Zaki, S. A. (2018). A review of artifacts in histopathology. Journal of Oral and Maxillofacial Pathology, 22(2), 279.

16. Sartor, R. B. (2006). Mechanisms of Disease: pathogenesis of Crohn's disease and ulcerative colitis. Nature Reviews Gastroenterology & Hepatology, 3, 390–407.

17. Tripathi, K., & Feuerstein, J. D. (2019). New developments in ulcerative colitis: latest evidence on management, treatment, and maintenance. Drugs Context, 8, 212572.

18. Moeinian, M., Ghasemi-Niri, S. F., Mozaffari, S., Abdolghaffari, A. H., Baeeri, M., Navaea-Nigjeh, M., & Abdollahi, M. (2014). Beneficial effect of butyrate, Lactobacillus casei and L-carnitine combination in preference to each in experimental colitis. World Journal of Gastroenterology, 20(31), 10876-85.

19. Fatima, D., & Shaza, A. (2016). Therapeutic Effect of Olmesartan Medoxomile Alone and in Combination with Sulfasalazine in Experimentally Ulcerative Colitis Model in Rats Change of body weight. International Journal of Clinical Pharmacology and Toxicology, 8(2), 97–106.

20. Kumar, V. S., Rajmane, A. R., Adil, M., Kandhare, A. D., Ghosh, P., & Bodhankar, S. L. (2014). Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. Journal of Biomedical Research, 28(2), 132-45.

21. Khan, I., Ullah, N., Zha, L., Bai, Y., Khan, A., Zhao, T., Che, T., & Zhang, C. (2019). Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBS): Cause or Consequence? IBS Treatment Targeting the Gut Microbiome. Pathogens, 8(3), 126.

22. Ulfig, A., Leichert, L. I. (2020). The effects of neutrophil-generated hypochlorous acid and other hypohalous acids on host and pathogens. Cellular and Molecular Life Sciences, 78, 385–414.