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RESEARCH ARTICLE

Evaluation of the effect of *Aegle marmelos* and *Punica granatum* in a murine model of dextran sulfate sodium-induced acute colitis

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ABSTRACT

Background: Ulcerative colitis (UC) is a debilitating condition associated with many complications. The current treatment regimen includes the use of anti-inflammatory agents such as sulfasalazine and corticosteroids which are associated with multiple adverse effects due to which, patients of UC tend to have a reduced quality of life from continuing disease activity. Textbooks on Ayurveda, the Indian traditional system of medicine, have described plants with property to provide strength to the body tissues and protecting them from damage. **Aims and Objectives:** We planned to study the effect of two such plants, *Aegle marmelos* and *Punica granatum* individually and in combination regimens in a murine model of dextran sulfate sodium (DSS)-induced colitis. **Materials and Methods:** In Phase I, 42 Swiss albino mice were divided into seven groups (n = 6/group) and treated as follows: Normal control and disease control (normal saline), positive control (sulfasalazine - 100 mg/kg) and the four test groups with *A. marmelos* - 0.39 g/kg/day and 0.78 g/kg/day and *P. granatum* - 5.20 g/kg/day and 10.40 g/kg/day. In Phase II, 30 mice were divided into five groups (n = 6/group) as follows: Normal control, positive control, *A. marmelos* - 0.78 g/kg/day + *P. granatum* - 10.40 g/kg/day, *A. marmelos* - 0.78 g/kg/day + sulfasalazine - 50 mg/kg, and *P. granatum* 10.40 g/kg/day + sulfasalazine - 50 mg/kg. All groups received treatment from day 1 to 14 and the inducing agent DSS from day 8 to 14 (except normal control). Variables assessed were colon length, colon weight-by-length ratio (analyzed using one-way ANOVA) and disease activity index, colitis macroscopy, and colon histopathology (analyzed by Kruskal–Wallis test). A value of $P < 0.05$ was considered to be statistically significant. **Results:** The high dose of *A. marmelos* caused a significant improvement in all the variables ($P < 0.05$) and was comparable to positive control ($P > 0.05$) while the high dose of *P. granatum* only decreased the colon weight-by-length ratio. The plant drug combination significantly improved all variables except histopathology. *A. marmelos* + sulfasalazine - 50 mg/kg combination was not significantly different compared to positive control on all variables ($P > 0.05$), whereas *P. granatum*+sulfasalazine - 50 mg/kg significantly improved all variables ($P < 0.05$) except the colitis score and histopathology. **Conclusion:** The combination of *A. marmelos* + *P. granatum* and that of each plant drug with low dose sulfasalazine were as effective as the standard dose of sulfasalazine in the model of DSS induced colitis.

KEY WORDS: *Aegle marmelos; Punica granatum*; Ulcerative Colitis; Dextran Sulfate Sodium

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INTRODUCTION

Ulcerative colitis (UC) is one of the two main forms of inflammatory bowel disease, the other being Crohn's disease. It is a chronic, relapsing, remitting, and inflammatory condition that affects an individual throughout life.[1,2] In UC, inflammation is continuous, superficial - involving only the mucosal layer

and confined to the colon and rectum unlike that in CD, where the inflammation is patchy and can affect any part of the gastrointestinal tract.[3] Both exogenous (composition of normal intestinal flora) and endogenous host factors (intestinal epithelial cell barrier function/innate and adaptive immune function) interact to cause a chronic state of deregulated mucosal immune function, further modified by environmental factors.[4]

The trend of inflammatory bowel disease has been increasing in the world and a recent study suggests that India has the highest incidence and prevalence of UC in Asia.[5,6] A study conducted in the Punjab state of India, observed the incidence of UC to be 6.0/100,000.[7]

UC is a debilitating condition associated with many complications. It is an important known risk factor for the development of colon cancer. The management of UC does not aim at curing the patient from the illness but mainly at attenuating the symptoms and improving the daily life of the patient suffering with it. The current treatment regimen includes the use of anti-inflammatory agents such as sulfasalazine and corticosteroids.[3] These drugs reduce the inflammation but are associated with multiple adverse effects due to which, patients of UC tend to have a reduced quality of life from continuing disease activity. Ayurveda, the Indian traditional system of medicine, has been used by various physicians to treat various types of gastrointestinal disorders. Ayurveda textbooks have described some plants with "*Balya*" property, meaning the property to provide strength to the body tissues and protecting them from damage. Some of the Ayurvedic plants mentioned to be having Balya property are *Tinospora cordifolia*, *Aegle marmelos,* and *Punica granatum*. Of these, *A. Marmelos* and *P. granatum* have been found effective for the treatment of various gastrointestinal disorders such as diarrhea and dysentery.

A. marmelos, commonly known in India as Bael in Hindi and Bilva in Sanskrit, belongs to the family Rutaceae. Various parts of the plant are used in Ayurveda and Unani medicine for the treatment and prevention of gastrointestinal disorders such as dysentery, diarrhea, and inflammation of the gastrointestinal tract.[8]

P. granatum, popularly known in India as Anaar (Hindi), belongs to the family Punicaceae. It is also used extensively in Ayurveda for the treatment of various disorders such as diarrhea and ulcers due to its astringent property.[9]

A thorough literature search revealed that very few studies demonstrating the effect of these Ayurvedic plants on large bowel diseases have been published, and no study has evaluated the effect of these plants in an experimental animal model of acute colitis using sulfasalazine as a comparator.[8-11] Moreover, none of the studies had tested the effectiveness of these Ayurvedic plants as an adjunct to the current standard allopathic treatment.

We, therefore, decided to study the effects of *A. marmelos* and *P. granatum* in the animal model of dextran sulfate sodium (DSS)-induced acute colitis,[1,12,13] as compared to sulfasalazine. In addition, we also assessed the effects of the combination of *A. marmelos* and *P. granatum* and the individual plant drugs as an adjunct to a lower dose of sulfasalazine.

MATERIALS AND METHODS

All the experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee (Approval Number: AEC/08/2014) of Seth G.S. Medical College and K.E.M. Hospital, Mumbai, which is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg.No: 60/PO/ReBi/S/99/CPCSEA).

Study Drugs and Chemicals

DSS (molecular weight: 36,000–50,000 Daltons) and the positive control sulfasalazine were purchased from MP Biomedicals, USA. The aqueous extracts of the study drugs were procured from Konark Herbals and Healthcare. Dry extract of *A. marmelos* (extractive value 10% w/w) was prepared using the unripe fruit while the rind of *P. granatum* was used to prepare its dry extract (extractive value 20% w/w). Both the extracts were subjected to qualitative analysis which included physicochemical tests, assessment for heavy metals, and microbiological tests and these were stored in a cool and dry place during the study period.

Animals and Treatment

Swiss albino mice of either sex weighing 18–22 g and >6 weeks in age were used. Animals were housed at the center for animal studies of the institute in air-conditioned rooms at a temperature of 22 ± 3°C and relative humidity of 30–70% under a 12 h dark and light cycle. Food and water were provided *ad libitum*.

The study was carried out in two phases. In Phase I, 42 mice were divided into seven groups (n = 6/group) as follows: Group I (normal control): Normal saline, Group II (disease control): Normal saline, Group III (positive control): Sulfasalazine (100 mg/kg/day), Group IV: *A. marmelos*: low dose (0.39 g/kg/day), Group V: *A. marmelos*: High dose (0.78 g/kg/day), Group VI*: P. granatum*: Low dose (5.2 g/kg/day), and Group VII: *P. granatum*: High Dose (10.4 g/kg/day) + 3% DSS

Doses of *A. marmelos* (0.39 g/kg/day and 0.78 g/kg/day) and *P. granatum* (5.2 g/kg/day and 10.4 g/kg/day) were extrapolated from the human doses mentioned in standard Ayurveda textbooks[14,15] and then converted to that of mice according to the Paget and Barnes dose conversion table.[16]

The experimental animals were administered the test drugs for 15 days. They were pre-treated with the test drugs/normal saline orally for 7 days. From day 8 onward in addition to the test drugs, all the groups (except Group I) were given standardized 3% DSS in drinking water till day 14. From day 8 to 14, the body weight of mice was checked daily with the help of a digital weighing machine and the stool was checked daily for consistency and blood to estimate the disease activity index (DAI). On day 15, mice were sacrificed and the whole colon was isolated. Following this, the length and weight of the colon were measured, and the colon was subjected to macroscopic and histopathological examination to assess the severity of colitis.

In Phase II, 30 Swiss albino mice were divided into five groups (n = 6/group) as follows: Group I (disease control): Normal saline, Group II (positive control): Sulfasalazine (100 mg/kg/day), Group III (plant drug combination): *A. marmelos* (0.78 g/kg/day) with *P. granatum* (10.4 g/kg/day), Group IV: *A. marmelos* (0.78 g/kg/day) + Sulfasalazine (50 mg/kg/day), and Group V: *P. granatum* (10.4 g/kg/day) + Sulfasalazine (50 mg/kg/day)

In this phase, the doses of *A. marmelos* and *P. granatum* were decided based on the dose which showed the best response in the Phase I. Sulfasalazine was administered in the dose of 50 mg/kg/day, which is half the standard dose of the drug. Pre-treatment with the test drugs, administration of 3% DSS, and assessment of variables were done as stated in Phase I.

Measurement of DAI[17]

The DAI for each animal was calculated on days 8, 10, 12, and 14 by summing up the score of three variables [Table 1] and the mean DAI of a group for that day was determined.

Measurement of Colon Length and Colon Weight

The isolated colon was rinsed with a sterile phosphate buffer solution to clear the fecal matter. The length and the weight of each colon were measured using a Vernier caliper and a digital weighing scale, respectively, and the colon length/weight ratio was calculated.[18]

Macroscopic Examination of Colitis Severity

The colons were longitudinally incised, examined for the presence of ulcers and the severity of colitis was assessed macroscopically using the following scoring system: [19]

0 - No ulcer and no inflammation; 1 - Local hyperemia without ulceration; 2 - Ulceration without hyperemia; 3 - Ulceration and inflammation at one site only; 4 - Two or more sites of ulceration and inflammation; and 5 - Ulceration extending >2 cm.

Table 1: DAI

Score	Weight loss (%)	Stool consistency	Blood in stool
0	None	Normal	Negative
1	1–5	-	-
2	6–10	Loose stool	Positive
3	11–15	-	-
4	>15	Diarrhea	Gross rectal bleeding

DAI: Disease activity index

Table 2: Histology scoring system for colon samples

Feature scored	Score	Description
Inflammation severity	0	None
	1	Mild
	2	Moderate
	3	Severe
Inflammation extent	0	None
	1	Mucosa
	2	Mucosa and submucosa
	3	Transmural
Crypt damage	0	None
	1	1/3 of crypt damaged
	2	2/3 of crypt damaged
	3	Crypts lost, surface epithelium intact
	4	Crypts lost, surface epithelium lost
Percent area of involvement (%)	0	0
	1	1–25
	2	26–50
	3	51–75
	4	76–100

Histopathological Examination of Colon

The longitudinally incised colons of each mouse were individually rolled using the "Swiss roll Technique" [Figure 1].[20] The rolled colon samples were fixed, embedded in liquid paraffin, sectioned, and stained with hematoxylin and eosin. The sections were microscopically examined for histopathologic changes using a validated scoring system as described previously [Table 2]. The histopathology score was determined by multiplying the sum of the scores of the three histological features with the score for the percent area of involvement. Thus, the minimum and maximum scores that could be obtained were 0 and 40, respectively.[21,22]

Statistical Analysis

All the results were expressed as mean ± SD. Data analysis was performed using GraphPad InStat software (GraphPad, San Diego, CA). Statistical comparisons were made between drug-treated groups and colitis control animals. Data of parametric variables (colon length and colon weight-by-length) were analyzed using one-way ANOVA followed by

Dunnett's *post hoc* analysis. The non-parametric variables (DAI, colitis macroscopic score, and the colitis histology score) were analyzed by Kruskal–Wallis test followed by *post hoc* Dunn's test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Phase I

The DAI scores [Table 3] of the sulfasalazine group on days 10, 12, and 14 were significantly lower ($P < 0.01$) than that of the disease control group. The high dose of *A. marmelos* showed a trend to decrease the DAI on day 10 and significantly reduced the DAI on day 12 ($P < 0.05$) as compared to the disease control but could not maintain the reduced DAI till day 14. The DAI on day 12 with the high dose of *A. marmelos* was significantly more than that observed with sulfasalazine. The low dose of *A. marmelos* and both the doses of *P. granatum* did not cause a significant decrease in the DAI.

As shown in Table 4, the mean colon length was significantly higher in the sulfasalazine group and the high dose of *A. marmelos* group as compared to the disease control ($P < 0.05$). Furthermore, the sulfasalazine group and *A. marmelos* high-dose group were not significantly different from each other ($P > 0.05$).

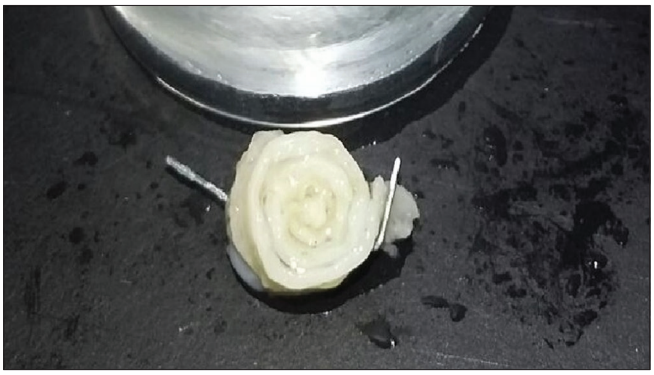

Figure 1: Swiss roll technique for histopathology of colon

Similarly, the groups which received sulfasalazine and high doses of *A. marmelos* and *P. granatum* exhibited a significantly lesser colon weight-by-length ratio ($P < 0.05$) as compared to the disease control. The colon weight-by-length ratios observed with the lower doses of *A. marmelos* and *P. granatum* were not significantly different from the disease control. The colon weight-by-length ratios observed with the higher doses of the plant drugs were not significantly different from the sulfasalazine group.

The macroscopic colitis score was significantly lesser in sulfasalazine group and the high dose of *A. marmelos* group as compared to the disease control group ($P < 0.05$) and these two groups were not significantly different from each other.

The histopathology scores of the group which received sulfasalazine and that of the group which received the high dose of *A. marmelos* were found to be significantly lower ($P < 0.05$) as compared to the disease control group. Once again, the high dose of *A. marmelos* group was not significantly different from the positive control sulfasalazine ($P > 0.05$) [Figure 2]. The low doses of both the plant drugs and the high dose of *P. granatum* did not cause a significant reduction in the colitis and the histopathology scores as compared to the disease control.

In Phase II, it was observed that the group receiving the plant drug combination and that receiving *A. marmelos* + sulfasalazine showed a significantly lower DAI ($P < 0.05$) as compared to the disease control [Table 5] and it was not significantly different from the positive control. The group receiving *P. granatum* + sulfasalazine combination also reduced the DAI significantly as compared to the disease control but could not maintain the reduction in the disease activity till day 14.

The plant drug combination and *A. marmelos*+sulfasalazine group increased the mean colon length, decreased the colon weight-by-length ratio, and decreased the macroscopic colitis score significantly ($P < 0.05$) when compared to the disease control group [Table 6] and were comparable to the

Table 3: DAI in various experimental groups (Phase I)

Group No.	Study groups	Day of DAI assessment		
		Day 10	Day 12	Day 14
I	Normal control	0.0	0.3±0.67	0.5±0.71
II	Disease control	3.0±1.78@	7.5±1.38@	9.167±1.6@
III	Positive control (Sulfasalazine 100 mg/kg)	0.5±0.54**	2.16±0.98**	3.33±1.86**
IV	*A. marmelos* (0.39 g/kg/day)	2.33±1.07	6.83±0.75	9.67±1.75
V	*A. marmelos* (0.78 g/kg/day)	1.5±0.67	3.67±0.52*	5.0±1.26
VI	*P. granatum* (5.20 g/kg/day)	1.67±1.91	6.67±1.37	8.83±1.72
VII	*P. granatum* (10.40 g/kg/day)	1.16±1.47	5.67±0.82	6.67±1.75

@P<0.001 versus normal control; *P<0.05, **P<0.01 versus disease control; #P<0.05 versus positive control. Analysis was done using Kruskal–Wallis test followed by *post hoc* Dunn's test. *A. marmelos*: *Aegle marmelos*, *P. granatum*: *Punica granatum*, DAI: Disease activity index

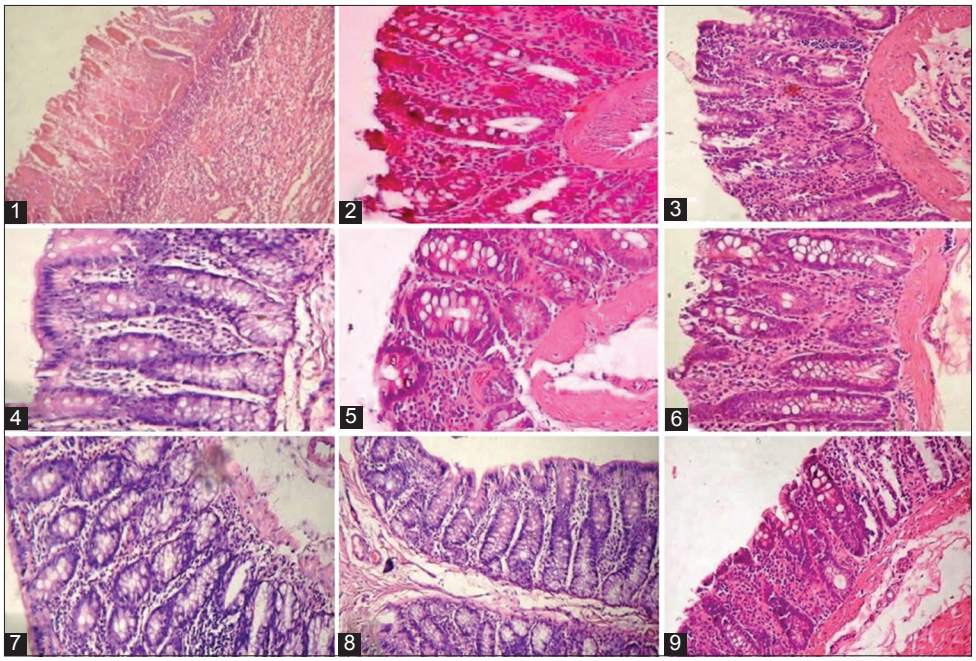

Figure 2: Histopathological effects of *Aegle marmelos* (AM) and *Punica granatum* (PG) on the dextran sulfate sodium (DSS - 36000-50000 MW)-induced acute colitis (×10). (1) Disease control treated with DSS showing crypt distortion along with hemorrhage and transmural leukocytic infiltration, (2) positive control sulfasalazine (100 mg/kg) with DSS showing minimal lymphocytic infiltration in mucosa, (3) low dose of AM with DSS showing cryptitis and dense lymphocytic infiltration in mucosa and submucosa, (4) high dose of AM given with DSS showing minimal crypt distortion and mucosal leukocytic infiltration, (5) low dose of PG with DSS showing prominent crypt distortion and dense leukocyte infiltration, (6) high dose of PG with DSS showing minimal crypt damage and leukocyte infiltration, (7) combination of the high doses of AM and PG along with DSS showing minimal leukocyte infiltration with normal crypts, (8) high dose of AM with sulfasalazine (50 mg/kg) along with DSS showing near-normal crypt structure and minimal mucosal leukocyte infiltration, (9) high dose of PG with sulfasalazine (50 mg/kg) along with DSS showing minimal crypt damage and infiltration in mucosa

Table 4: Effect of the test drugs on the colon length, colon weight/length, macroscopic grading of colitis, and histopathology score (Phase I)

Group No.	Test group	Colon length (cm)	Colon weight/length (mg/cm)	Macroscopic grading of colitis	Histopathology score
I	Normal control	7.76±0.27	22.29±1.31	0.20±0.48	1.20±1.31
II	Disease control	5.82±0.66$^{@}$	33.02±4.31$^{@}$	4±0.90$^{@}$	24.17±3.48$^{@}$
III	Positive control (Sulfasalazine 100 mg/kg)	7.78±0.24**	22.34±1.03**	1±0.89**	5.5±0.54**
IV	*A. marmelos* (0.39 g/kg/day)	6.39±0.54	28.29±1.90	2.5±1.05	14.5±6.31
V	*A. marmelos* (0.78 g/kg/day)	7.38±0.30**^{NS}	24.70±1.58**^{NS}	1.83±0.82*^{NS}	9.0±1.79*^{NS}
VI	*P. granatum* (5.20 g/kg/day)	6.38±0.92	28.28±4.29	3±0.55	12.33±8.64
VII	*P. granatum* (10.40 g/kg/day)	6.83±0.93	25.41±4.47**^{NS}	2.33±1.21	11.5±8.87

All results expressed in mean±SD, colon length and colon weight/length were analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. Macroscopic grading of colitis and the histopathology score were analyzed using Kruskal–Wallis test followed by Dunn's *post hoc* test. $^{@}P$<0.001 versus normal control; *P<0.05, **P<0.01, ***P<0.001 versus disease control; NS: Not significant versus positive control. *A. marmelos*: *Aegle marmelos*, *P. granatum*: *Punica granatum*

positive control group. *P. granatum* + sulfasalazine group caused a significant increase in the mean colon length and decrease the colon weight-by-length ratio as compared to the disease control but was not comparable to the positive control. Further, it did not cause a significant decrease in the macroscopic colitis score.

The histopathology scores revealed that only *A. marmelos* + sulfasalazine group was comparable to the positive control [Table 6].

DISCUSSION

The present study evaluated the effect of *A. marmelos* and *P. granatum* individually and in combination with each other and with sulfasalazine in a murine model of DSS-induced colitis. This model has many advantages over the other animal models. For instance, the clinical features seen in the acute phase of DSS-induced colitis include loss of weight, diarrhea, occult blood in stools, and frank bleeding per rectum are reminiscent of the clinical characteristics of human colitis. Moreover,

Table 5: DAI in various experimental groups (Phase II)

Group No.	Study groups	Day of DAI assessment		
		Day 10	Day 12	Day 14
I	Disease control	3±1.78	7.5±1.38	9.16±1.60
II	Positive control (Sulfasalazine 100 mg/kg)	0.5±0.44**	2.16±0.99**	3.16±1.72**
III	*A. marmelos*+*P. granatum*	0.83±1.17*	2.33±1.6* ^{NS}	4.16±2.13*^{NS}
IV	*A. marmelos*+Sulfasalazine (50 mg/kg)	0.56±0.54*^{NS}	2.5±1.51*^{NS}	3.83±1.72*^{NS}
V	*Punica granatum*+Sulfasalazine (50 mg/kg)	0.67±1.21*^{NS}	2.67±1.82*^{NS}	5.33±2.42

*P<0.05, **P<0.01, versus disease control; NS - Not significant versus positive control using Kruskal–Wallis test followed by *post hoc* Dunn's test. *A. marmelos*: *Aegle marmelos*, *P. granatum*: *Punica granatum*, DAI: Disease activity index

Table 6: Effect of various combinations using AG and PG on the colon length, colon weight/length, macroscopic grading of colitis, and colitis histopathology score in mice with DSS-induced acute colitis (Phase II)

Test group	Colon length (cm)	Colon weight/ length (mg/cm)	Macroscopic grading of colitis (0–5)	Colitis histopathology score (0–40)
Disease control	5.8±0.65	33.21±4.61	3.63±0.61	17.83±9.18
Positive control (Sulfasalazine 100 mg/kg)	7.83±0.14**	22.52±1.13**	1.17±0.69**	5.6±0.41**
AM (0.78 g/kg/day)+PG (10.4 g/kg/day)	7.26±0.47**^{NS}	24.93±2.29*^{NS}	1.67±0.54* ^{NS}	10.33±3.44
AM (0.78 g/kg/day)+sulfasalazine (50 mg/kg)	7.33±0.33**^{NS}	24.45±1.31*^{NS}	1.5±0.75*^{NS}	8.66±2.42*^{NS}
PG (10.40 g/kg/day)+sulfasalazine (50 mg/kg)	6.98±0.92*$^{\#}$	26.70±4.21*$^{\#}$	1.83±0.54	12.33±3.882

AM: *Aegle marmelos*, PG: *Punica granatum*, DSS: Dextran sulfate sodium. All results expressed in mean±SD; colon length and colon weight/length were analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. Macroscopic grading of colitis and the histopathology score were analyzed using Kruskal–Wallis test followed by Dunn's *post hoc* test. *P<0.05, **P<0.01, ***P<0.001 versus disease control; NS - Not significant and $^{\#}P$<0.05 versus

histological features of experimentally induced colitis in mice such as degeneration of epithelium, infiltration of inflammatory cells in the mucosa and submucosa, inflammation of the crypts, and necrotic changes[3] also closely resemble the histological features of colitis found in humans. In the present study, it was found that the high dose of *A. marmelos* had significantly improved all the variables ($P < 0.05$). Its effects on all variables were comparable to the positive control group except the DAI which was significantly more than the positive control. In the high dose of *P. granatum* group, only colon weight-by-length ratio was significantly different from the disease control group. Since the higher dose of *A. marmelos* had caused a significant improvement in all the variables and the higher dose of *P. granatum* showed a decrease in the weight/length ratio and a trend to improve the other variables including the DAI it was of interest to study the effect of the combination of these plant drugs. It was observed that the plant drug combination not only reduced the disease activity but also caused an improvement in all the other variables except the histopathology. The combination of the high dose of *P. granatum* + sulfasalazine reduced the colon length and colon weight-by-length ratio and the disease activity but did not have any effect on the colitis score or histopathology. Interestingly, the combination of the high dose of *A. marmelos* + sulfasalazine caused a significant improvement in all the variables assessed in the study and was comparable to sulfasalazine - 100 mg/kg. The results indicate that there was an additive effect when the plant drugs were combined with each other or with the lower dose of sulfasalazine.

The results observed with a high dose of *A. marmelos* were similar to those obtained in studies carried out by other researchers although these studies used other experimental models and did not evaluate the effects of the aqueous extracts of *A. marmelos*.[10,23] For instance, in the study was conducted by Gautam *et al*., the ethanolic extract of the fruit pulp of *A. marmelos* decreased the tissue damage and inflammation in rats suffering from acetic acid-induced colitis.[10] Another study conducted by Ghatule *et al*. demonstrated the curative effects of the ethanolic extract of *A. marmelos* against 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced experimental colitis in rats by showing antibacterial activity and decreased colonic mucosal damage.[23] However, unlike our study, in these studies, the effects of *A. marmelos* were not compared with a positive control like sulfasalazine. The fruit pulp extract of *A. marmelos* has multiple bioactive compounds such as phenolics, tannins, and flavonoids which have been demonstrated to have anti-inflammatory, antimicrobial, and immunomodulatory properties. It is possible that the protective effect against colitis exhibited by the plant is due to these bioactive compounds.[23]

The results obtained with a high dose of *P. granatum* were similar to those observed in studies conducted by other researchers, however, as observed with *A. marmelos*, none of these studies evaluated the aqueous extract of *P. granatum*.[24,25] For instance, Singh *et al.* have reported the antiulcerative effect of the methanolic extract of *P. granatum*

flowers in the DSS-induced colitis model flowers.[24] On the other hand, Kalangottil *et al.* have reported the anti-inflammatory and antioxidant effect of the hydroalcoholic extract of *P. granatum* rind against acetic acid and TNBS-induced UC in rats.[25] The fruit rind of *P. granatum* is known to be rich in ellagitannins, which releases ellagic acid on hydrolysis. Ogawa *et al.* have documented that oral administration of the microspheres of ellagic acid provides protection against DSS-induced UC in rats.[26] Marín *et al*. have also reported the anti-inflammatory effect of ellagic acid in the same model.[11] However, as was the case with studies evaluating *A. marmelos*, the effects of *P. granatum* were not compared with a positive control. Ellagic acid has also been found to alleviate acute colitis by its modulatory effect on inflammatory mediators such as interleukin 6, interferon-gamma, and tumor necrosis factor-alpha.[12] The fruit rind of *P. granatum* has been shown to contain flavonoids and alkaloids, which have been found to be responsible for reducing the production of autacoids and prostaglandins, thereby reducing the severity of inflammatory response.[27] We cannot compare our results of the combination regimens to those conducted by other researchers as the other studies have not evaluated the effect of test drug combinations.

The present study is the first study to examine the effects of the aqueous extracts of *A. marmelos* and *P. granatum*, individually, in combination and as an adjunct to a low dose of sulfasalazine in an animal model of acute colitis. One of the strengths of this study was the use of a positive control like sulfasalazine which has not been used in similar studies conducted by other researchers. It is well known that the use of sulfasalazine is associated with various side effects which include depression in young males, thrombocytopenia, headache, dizziness, and hemolytic anemia.[28] Further, it also causes inhibition of dihydropteroate synthase which can lead to megaloblastic anemia.[29] Hence, we felt it would be worthwhile to study the effects of the high doses of the plants in combination with half the sulfasalazine dose as such a combination could possibly have fewer adverse effects without compromising the efficacy. Thus, our study, though preliminary, involved multiple variables for evaluation to get a comprehensive picture. A limitation of the study was that the effect of these extracts was tested only on an acute model of colitis and not on a chronic pre-clinical model. The probable chemical mechanisms involved were not studied, which can be explored by researchers in the future using some objective parameters.

CONCLUSION

Our study has demonstrated that the combination of *A. marmelos* + *P. granatum* and that of *A. marmelos* with low-dose sulfasalazine was as effective as high-dose sulfasalazine in the model of DSS-induced colitis. Further studies to evaluate the effect of the plant drugs in animal models of chronic colitis need to be carried out. Furthermore, the effect of these combinations as treatment options in patients of UC who are either intolerant to the standard dose of sulfasalazine or cannot tolerate even low doses of sulfasalazine needs to be studied in clinical trials.

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