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PROTECTIVE EFFECT OF *PICCHA BASTI* IN PREVENTIVE AND THERAPEUTIC MODEL OF ACETIC ACID INDUCED ULCERATIVE COLITIS IN C57BL/6 MICE

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ABSTRACT

Ulcerative colitis is equated with *Pitta-Kaphaja Grahani* in Ayurveda with *Piccha basti* as the preferred treatment. Hence the present study was undertaken for evaluating of *Piccha basti* in experimental colitis.

Experimental colitis was induced in C57BL/6 mice with 1ml intra colonal 2% acetic acid (1 mL). *Piccha basti* was administered in both preventive and therapeutic model for 8 and 16 days. Colitis score, colon weight/length ratio, Disease Activity Index along with mucosal anti-oxidants-SOD, CAT, GSH, LPO, and myeloperoxidase activity, TNFα, IL-6, IL-1β and histopathological examinations were assessed.

Results: Remarkable and statistically significant changes occurred in both macroscopical and cytokine parameters after acetic acid administration in positive control group in comparison to normal control in both preventive and therapeutic models except for IL6 which showed decrease in therapeutic model. The altered parameters were found to be significantly reversed by *Piccha basti* (*Piccha basti*) of both 8 & 16 days duration and standard treatment (Mesalmine enema) except that IL6 lowering was non-significant (p0.05) in *Piccha basti* 8 days group. Anti-oxidant parameters and MPO did not exhibit significant changes among the groups. Histopathology examination showed moderate reversal of the cytoarchitectural disturbances in PB-8 and 16 days groups in preventive model and good reversal in standard group. Reversal of the histopathological changes were found to be much better in therapeutic model.

Conclusion: Based on the results it is concluded that *Piccha basti* for 8 and 16 days in both preventive and therapeutic models has good anti colitis effect. The effect is higher in therapeutic model and corroborated *Piccha basti* clinical indication of colitis like conditions.

Keywords: Ulcerative colitis, *Piccha basti*, C57BL/6 mice, Mesalamine enema

INRTODUCTION

Worldwide, the highest incidence and prevalence of inflammatory bowel diseases are seen in Northern Europe and North America. Inflammatory bowel diseases are closely linked to a westernised environment and lifestyle. Ulcerative colitis has an incidence of 9 to 20 cases per 100,000 persons per year **[1]**. A population based study from India reported a crude prevalence rate of 44.3 per 100,000 inhabitants, which gave a crude incidence of 6.02 cases per 100,000 inhabitants. This was the first population based study from India reporting on the incidence and prevalence of Ulcerative colitis, the disease frequency is not much less that reported from Europe and North America **[2]**.

Ulcerative colitis is a chronic, idiopathic inflammatory disease that affects the colon, most commonly afflicting adults aged 30-40 years and resulting in disability **[3, 4]**. It is characterised by relapsing and remitting mucosal inflammation, starting in the rectum and extending to proximal segments of the colon **[5]**. Aminosalicylates are the main choice of treatment for mild to moderate Ulcerative colitis, topical and systemic steroids can be used to treat Ulcerative colitis flares, while immunosuppressants and biological drugs are used in moderate to severe disease. Colectomy is needed in up to 15% of patients with Ulcerative colitis **[6]**. Drugs such as the oral contraceptives, hormone replacement therapy and non-steroidal anti-inflammatory drugs, have all been associated with an increased risk of Ulcerative colitis **[7-13]**.

Ulcerative colitis majorly presents with bloody diarrhoea, rectal bleeding, mucus passage and crampy abdominal pain. Patients with proctitis usually pass fresh blood or blood-stained mucus either mixed with stool or streaked onto the surface of a normal or hard stool. Some patients pass frequent, small volume fluid stools while others are constipated and pass pellet stools. *Pitta-Kaphaja Grahani* shows symptoms like passing of undigested food with constipation and loose stools at frequent intervals, sour burps, anorexia, abdominal discomfort and weakness.

The management of Ulcerative colitis includes use of 5-ASA agents like Sulfasalazine, Olsalazine, Glucocorticoids, Salicylates, topical mesalamine enemas and suppositories. The side effects are numerous including fluid retention, abdominal striae, fat redistribution, hyperglycaemia, emotional disturbances, etc. Surgery is the ultimate option to maintain Ulcerative colitis, when it re-occurs due to failure of the therapeutic maintenance.

Need for the Study

In *Ayurveda*, *Piccha basti* is one of the very effective and result oriented treatment for Ulcerative colitis which is also very cost effective. There is also relief found clinically with respect to signs and symptoms.

In Ulcerative colitis, there is inflammation in the colon and *Basti dravya* comes in direct contact with the colon. *Piccha basti* is indicated in the treatment of *Grahani*. *Changeryadi Ghrita* is indicated in *Arsha*, *Grahani*, *Atisara*, *Pravahika* and *Mutrakrichra*.

Since there are no validated animal studies to prove the mechanism of action of *Piccha basti*, this study was undertaken in mouse model of Ulcerative colitis to ascertain the effect of *Piccha basti* administered both in preventive and

therapeutic model on experimental Ulcerative colitis along with an aim to identify probable mode of action. The effort is to provide experimental evidence to the clinical efficacy of the procedure.

MATERIALS AND METHODS

The study was carried out in College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru with the approval of IEAC of the institution (Approval No. DSU/CR/IAEC/40/2019-20 dated 14/12/2019).

Animals

Sixty female C57BL/6 mice weighing 25-30g were purchased from Raghavendra enterprises, Bengaluru and maintained at the faculty of Dayananda Sagar College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru. The mice were housed in plastic ventilated cages under a 12-hour light-dark cycle, with room temperature (RT) maintained at 22°C, humidity at 55%, and food and water available ad libitum. The study was conducted after obtaining the permission of the Institute's Animal Ethics Committee (Approval No. DSU/CR/IAEC/40/2019-20 dated 14/12/2019)

Induction of colitis

Colitis was induced in mice by intra-colonic administration of 2% acetic acid (1 mL) through a lubricated catheter under low-dose ether anaesthesia. The instillation site was about 5 cm from the anal verge into the rectum. Mice were maintained in trendelenburg position for 30 sec to prevent the leakage of the acid.

Study Design

Two models, preventive and therapeutic model, were employed in the study with a total of 10 groups (5 groups in each model). Sixty animals were randomly divided into ten experimental groups (n=6 per group). Full details are provided in **Table 1**.

Assessment Criteria

1. Macroscopic and histological evaluation of colonic damage: A 10cm segment of the distal colon was removed for the morphological study. Colonic mucosa damage was assessed according to the standard scoring system as follows: 0 = normal mucosa; 1 = localized hyperemia but no erosions, ulcers, or scars; 2 = linear ulcer or scar with inflammation at one site >2 mm but <5 mm; 3 = two or more sites of ulceration and/or inflammation, each up to 5 mm; 4 = two or more major sites of inflammation and ulcerations >5 mm each or one major site of inflammation extending >1 cm along the length of the mucosa **[14]**.
2. Colon weight/length ratio (g/cm): After sacrificing the animals colons were removed, gently flushed with ice-cold normal saline placed on ice cold plate, cleaned of fat and mesentery and blotted on filter paper to dry lightly. Each colon was weighed and its length was measured. It was used as a parameter to assess the degree of colon oedema which reflects the severity of colitis.
3. Disease Activity Index (DAI) analysis: The DAI was determined at the end of the treatment period using the DAI scoring system **[15]**. The scores for stool consistency and occult blood for each mouse added and then given a DAI score for each mouse. Each score was determined as follows: stool consistency (0 and 1: normal, 2 and 3: loose stool, 4: diarrhoea) and stool blood (0: negative, 1: ±, 2: +, 3: ++, 4: gross).

4. Estimation of colonic mucosal antioxidants (SOD, CAT, GSH, LPO) and inflammatory marker (MPO).
5. Estimation of TNFα, IL-6 and IL-1β using ELISA.
6. Histopathological investigations: Colon sections were fixed in 10% neutral buffered formalin then kept for 24 hours. Samples were then cut into several sections and embedded into paraffin wax blocks. Tissues were stained with hematoxylin and eosin and were mounted and observed microscopically for histopathological changes.

Statistical Methods

Data are presented as Mean ± SEM. The differences among the groups were assessed by One way ANOVA with Tukey's test as Post hoc test for parametric data and Kruskal–Wallis (KW) test with Dunn's test as Post hoc test for non-parametric data. A $p<0.05$ was considered statistically significant. SPSS version 26 was used for this purpose.

RESULTS

The data obtained in Preventive model are shown in **Table 2**. Analysis of the Disease Activity Index related parameters revealed remarkable and significant elevation in both rectal bleeding and stool consistency score. Bleeding was found to be fully reversed in standard and PB-16 days groups. In PB-8 days group also significant reversal was observed. The improvement in the changed faecal consistency was also remarkable in standard and PB-16 days groups ($p<0.001$); in PB-8 days group also significant improvement was observed ($p<0.01$). The mucosal damage score increased by more than fivefold in colitis control in comparison to the normal control. This elevation was remarkably attenuated in all the three viz Standard, PB-8 days ($p<0.001$) and PB-16 days ($p<0.0001$) groups with PB-16 day groups showing the best outcome. The colon wt/length ratio was found to be moderately elevated in colitis control in comparison to the normal control ($p<0.05$) and this increase was also found to be significantly lowered in all the three treated groups ($p<0.01$ to <0.001).

Analysis of the cytokine related data revealed significant but moderate elevation in TNF-α and IL-6 levels and decrease in IL-1 β levels in colitis control in comparison to the normal control group. The elevation observed in TNF-α ($p<0.05$) and IL-6 ($p<0.001$) level was found to be significantly reversed in all the treated groups. The decrease in IL-6 level was even less than normal control groups. The decrease observed in IL-1 β level was significantly reversed in standard and PB-16 days group and moderate but non-significant reversal was observed in PB-8 days group.

The anti-oxidant and oxidation related parameters did not exhibit any remarkable changes in the colitis control and in colitis treated groups.

In therapeutic model groups also the Disease Activity Index parameters were found to be remarkably elevated in colitis control group and reversed in colitis treated groups. The mucosal damage and colon wt/length ratio were also found to be elevated. The former was found to be remarkably reversed in all colitis treated groups ($p<0.0001$), PB-16 days group exhibited the best effect. The colon wt/length ratio though moderately reduced in standard and PB-8 days groups the decrease was found to be non-significant. However, in PB-16 days group significant reversal was observed ($p<0.001$). Cytokine related analysis showed significant decrease in TNF-

α level and significant elevation in IL-6 and IL-1 β level in colitis control in comparison to the normal control group.

The anti-oxidant and oxidation related parameters did not exhibit any remarkable changes in the colitis control and in colitis treated groups similar to that observed in preventive model.

Histopathological examination of the colon tissue showed normal histological structure, including the mucosa (lamina epithelialis, lamina muscularis mucosa and lamina propria), the submucosa, the muscular coat (inner circular and outer longitudinal muscle fibres) and the serosa in the negative control group in both preventive and therapeutic model. In Disease control group (Positive control group) in both preventive and therapeutic models marked disruption, degeneration of mucosal layer accompanied by inflammatory cell infiltration was observed. In therapeutic model the changes were severe with massive necrosis and desquamative changes in the epithelium with deformation of the colon glands. In standard group healed ulcerative lesions with regeneration of epithelium and with moderate inflammatory reaction was observed. In PB-8 days group regenerated epithelial cells, focal hyperplastic changes with hyperchromatic nuclei (regenerative signs) were observed along with infiltration of the inflammatory cells. In PB-16 days group better regenerated epithelial cells, replacement of the submucosa by aggregated and/or follicular hyperplastic lymphoid cells was observed. The reversal of the degenerative changes in *Piccha basti* groups moderate in preventive model and very good in the therapeutic model. Details are provided in **Figure 1**.

DISCUSSION

The study was designed and undertaken with a view to ascertain whether *Piccha basti* has preventive and therapeutic potential against experimental acetic acid induced colitis in C57BL/6 mice. For this purpose, different kinds of parameters were employed. Main among them is the effect seen on the healing of colitis though morphological and functional ability based parameters. Further as supportive evidence cytokine parameters in the colon tissue homogenate were also recorded. Apart from considering that free radical generation and anti-oxidant balance plays important role in this colitis model – representative parameters SOD, Catalase and Glutathione content and lipid peroxidation related parameters were also recorded. As conclusive support histopathological study of lesions was carried out. The results from these experimental groups were compared with a reference standard therapy administered by the same route.

Careful analysis of the results shows that Disease Activity Index comprising rectal bleeding and fecal consistency shown to be remarkably increased in disease control group in comparison to the normal control group indicating robust nature of the model employed. These parameters were remarkably reversed in both preventive and therapeutic *Piccha basti*. Bleeding was found to be completely stopped in reference standard and PB-16 days treatment. It has been shown in previous studies that the decrease in the disease activity is the reflection of the attenuation in the severity of the disease. It can be suggested that marked improvement in DAI is the result of clinical improvement and decrease in the colonic inflammation and necrosis which might have led decreased DAI. Rectal bleeding is likely to be dependent up on the colonic ulceration

and stool consistency and formation on the functional integrity of the colon. Marked improvement in the morphological parameters in the form of decreased mucosal damage and colon wt to length ratio (WL ratio) is indicative of structural improvement **[16, 17]** which might have contributed to the functional improvement as shown above. In this mucosal damage score indicating the status of mucosal layer improved remarkably higher improvement was in PB-16 days group this may be due to promoting the factors maintaining mucosal barrier and functions. CWL ratio changes were moderate and in this parameter, also significant improvement was observed in both treatment and standard groups. Factors promoting mucosal integrity are likely to be promoted by the test treatment.

The inflammatory process has been identified as the main contributing factor in the experimental colitis similar to its clinical counterpart. Several mechanisms have been shown to play role in this. It has been reported earlier **[16-17]**. In this condition there is increased expression of inducible enzymes like cox-2 and iNOs involved in the formation of prostaglandins and nitric oxide respectively. Since they can also influence formation of inflammation promoting cytokines; it may get reflected in lower concentration of cytokines. This was the case as marked decreased in IL-6 & TNF-α – content of the colonic tissue indicating decreased inflammatory response. Thus, it can be suggested that modulation of formation and functioning of these cytokines may be one the important mechanism of action of *Piccha basti*. It would be interesting to ascertain the effect of *Piccha basti* on expression of the cox-2 and iNOs as mentioned above. However, decrease in IL-1 β was observed and it was moderately restored. It is possible that this cytokine may be under other stronger influences like myeloperoxidase activity which might not have been influenced by *Piccha basti*.

It has also been shown that in acetic acid induced colitis increased expression of the gene linked to MMP-9 group of proteases responsible for the degradation of matrix leading to the structural damage seen in the colitis. Besides MMP-9 has the potential to activate a wide range of cytokines, chemokines, receptors, signalling molecules accelerating and multiplying the inflammatory response **[16, 17]**. It is possible that *Piccha basti* may be modulating the activity and expression of these enzymes. However, actual studies with *Piccha basti* are required to provide conclusive proof on the role of this important mediator.

Analysis of the data related to anti-oxidant parameters and parameters related to lipid peroxidation and MPO **[16]** shows that anti-oxidant parameters are only marginally affected indicating that they may not have prominent role in the observed beneficial effects of *Piccha basti*. Lipid peroxidation was only a moderately affected and its attenuation was also marginal ruling out modulation of anti-oxidant and oxidation linked parameters.

Finally histopathological studies confirm and corroborate the effect on structural, functional and cytokine related parameters providing strong evidence for the protective effect observed. It can be noted that the effect is better with longer duration of *Piccha basti* in therapeutic model.

Thus, all the above mechanisms seem to contribute to the beneficial effects observed with *Piccha basti* and provide strong experimental evidence for its clinical

efficacy. In addition, there may be role for the phytochemical constituents in the basti formulation which needs further studies.

Figure 1 shows the histopathological studies of the mice colon in both preventive and therapeutic model.

The gross macroscopic alterations on the mice colon in both preventive and therapeutic model is shown in **Figure 2**.

Table 1: Details of the study design

1. Preventive Model					
1.1	**Negative Control**	**1 mL of normal saline enema for 23 days**			**On Day 24 the mice were sacrificed. Their distal colonic segments were excised, cut open to expose the inner surface, washed thoroughly with normal saline, and assessed macroscopically. Sections of the distal colon were stored in 10% formalin for Histopathological studies and colons were processed for biochemical parameters.**
1.2	**Positive Control**	**1 mL of 0.9% w/v normal saline enema for 16 days**	**After the last dose the mice were fasted for 12 hours**	**1 mL of 2% v/v acetic acid through rectal route for 4 days**	
1.3	**Standard Control**	**50mg/kg w/v mesalamine enema for 16 days**			
1.4	**Sample 8 days**	***Piccha basti* (Human equivalent dose of 3.48 mg/kg b.w.) for 8 days**			
1.5	**Sample 16 days**	***Piccha basti* (Human equivalent dose of 3.48 mg/kg b.w.) for 16 days**			

2. Therapeutic Model					
2.1	**Negative Control**	**1 mL normal saline enema for 23 days**			**On Day 24 the mice were sacrificed. Their distal colonic segments were excised, cut open to expose the inner surface, washed thoroughly with normal saline, and assessed macroscopically. Sections of the distal colon were stored in 10% formalin for Histopathological studies and colons were processed for biochemical parameters.**
2.2	**Positive Control**	**1 mL of 2% v/v acetic acid through rectal route for 4 days**	**After the last dose the mice were fasted for 12 hours**	**1 mL of 0.9% w/v normal saline enema for 16 days**	
2.3	**Standard Control**			**50mg/kg w/v mesalamine enema for 16 days**	
2.4	**Sample 8 days**			***Piccha basti* (Human equivalent dose of 3.48 mg/kg b.w.) for 8 days**	
2.5	**Sample 16 days**			***Piccha basti* (Human equivalent dose of 3.48 mg/kg b.w.) for 16 days**	

Table 2: Effect of *Piccha basti* on different parameters recorded in preventive model of experimental acetic acid induced colitis

Parameters	**Different groups**				
	NC	**PC**	**SC**	**PB 8**	**PB 16**
Disease Activity Index (DAI) analysis					
Rectal bleeding	**0.00 ±0.00**	**1.15± 0.42**	**0.00 ±0.00**	**0.24± 0.12$^{\#}$**	**0.00 ±0.00**
Stool consistency	**0.18± 0.06**	**1.55± 0.51***	**0.20± 0.09 $^{\#\#\#\#}$**	**0.62± 0.24 $^{\#\#}$**	**0.14± 0.06 $^{\#\#\#}$**
Macroscopic and histological parameters					
Mucosal damage scores	**0.50± 0.55**	**4.66± 0.52***	**1.33± 0.52 $^{\#\#\#}$**	**1.50± 0.55 $^{\#\#\#}$**	**0.83± 0.41$^{\#\#\#\#}$**
Colon wt/l (mg/cm)	**83.8± 26.20**	**97.3± 31.26***	**68.41± 21.20$^{\#\#\#}$**	**77.87± 26.96$^{\#}$**	**63.6± 26.25$^{\#\#\#}$**
Cytokines					
TNF-α	**0.29± 0.08**	**0.67± 0.25***	**0.25± 0.10 $^{\#}$**	**0.26± 0.20 $^{\#}$**	**0.19 ±0.09$^{\#}$**
IL-6	**0.10± 0.02**	**0.20± 0.01*****	**0.07± 0.01$^{\#\#\#\#}$**	**0.07± 0.01$^{\#\#\#\#}$**	**0.09± 0.01 $^{\#\#\#\#}$**
IL-1 β	**0.96± 0.20**	**0.35± 0.01 *****	**0.45± 0.04$^{\#\#}$**	**0.63± 0.01 ^{ns}**	**0.35± 0.01 $^{\#\#\#}$**
Anti-oxidant and oxidation related parameters					
SOD ng/mL	**0.50± 0.01**	**0.54± 0.01^{ns}**	**0.53± 0.01^{ns}**	**0.51± 0.01^{ns}**	**0.51± 0.0^{ns}**
Catalase ng/mL	**0.46± 0.01**	**0.48± 0.01^{ns}**	**0.47± 0.01^{ns}**	**0.45± 0.01^{ns}**	**0.48± 0.01^{ns}**
GSH ng/mL	**0.51± 0.01**	**0.54± 0.01***	**0.53± 0.01^{ns}**	**0.51± 0.01^{ns}**	**0.52± 0.01^{ns}**
MPO ng/mL	**0.53± 0.0**	**0.52± 0.0****	**0.52± 0.0$^{\#\#}$**	**0.52± 0.0^{ns}**	**0.52± 0.0$^{\#}$**
LPO ng/mL	**0.50± 0.01**	**0.47± 0.01***	**0.473± 0.01$^{\#}$**	**0.484± 0.01^{ns}**	**0.48± 0.01^{ns}**

PC = Positive Control, NC = Negative Control, SC = Standard Control, PB 8 = Sample 8 days, PB 16 = Sample 16 days. Data are expressed as means ± SEM. N=6 for each group. *p < 0.05, **p<0.01, *** = p<0.001, **** = p<0.0001, ns = not significant for comparison of negative normal control to Positive colitis control. #p < 0.05, ## p<0.01, ### p<0.001, #### p<0.0001, ns = not significant for comparison of treatment groups with positive colitis control

Table 3: Effect of *Piccha basti* on different parameters recorded in therapeutic model of experimental acetic acid induced colitis

Parameters	Different groups				
	NC	PC	SC	PB 8	PB 16
Disease Activity Index (DAI) analysis					
Rectal bleeding	0.00 ± 0.00	2.89± 0.19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stool consistency	0.15± 0.05	2.56± 0.19****	0.40± 0.23$^{\#\#\#\#}$	0.67± 0.20$^{\#\#\#\#}$	0.35± 0.13$^{\#\#\#\#}$
Macroscopic and histological parameters					
Mucosal damage scores	0.33± 0.43	4.67± 1.86****	0.83± 0.43$^{\#\#\#\#}$	0.66± 0.53 $^{\#\#\#\#}$	0.33± 0.73$^{\#\#\#\#}$
Colon wt/l (mg/cm)	75.3± 8.61	98.11± 7.37***	85.6± 8.49^{ns}	82.42± 10.50 ^{ns}	67.37± 15.61$^{\#\#\#}$
Cytokines					
TNF-α	0.65± 0.23	0.27± 0.11*	0.39± 0.05 $^{\#}$	0.25± 0.15 $^{\#\#}$	0.33± 0.02$^{\#}$
IL-6	0.08± 0.06	0.18± 0.02*	0.09± 0.05 ^{ns}	0.12± 0.03^{ns}	0.07± 0.01^{ns}
IL-1 β	0.10± 0.01	0.72± 0.13***	0.57± 0.07^{ns}	0.54± 0.04^{ns}	0.32± 0.03$^{\#}$
Anti-oxidant and oxidation related parameters					
SOD ng/mL	0.54± 0.0	0.50± 0.0*	0.52± 0.01^{ns}	0.51± 0.01^{ns}	0.52± 0.0^{ns}
Catalase ng/mL	0.48± 0.01	0.46± 0.01^{ns}	0.47± 0.01^{ns}	0.45± 0.01^{ns}	0.45± 0.01ns
GSH ng/mL	0.54± 0.01	0.51± 0.0^{ns}	0.53± 0.01^{ns}	0.52± 0.01^{ns}	0.52± 0.01^{ns}
MPO ng/mL	0.52± 0.00	0.53± 0.00**	0.52± 0.00**	0.52± 0.00^{ns}	0.52± 0.00^{ns}
LPO ng/mL	0.47± 0.01	0.50± 0.01*	0.47± 0.01^{ns}	0.49± 0.00^{ns}	0.48± 0.01^{n}

PC = Positive Control, NC = Negative Control, SC = Standard Control, PB 8 = Sample 8 days, PB 16 = Sample 16 days. Data are expressed as means ± SEM. N=6 for each group. *p < 0.05, **p<0.01, *** = p<0.001, **** = p<0.0001, ns = not significant for comparison of negative normal control to Positive colitis control. #p < 0.05, ## p<0.01, ### p<0.001, #### p<0.0001, ns = not significant for comparison of treatment groups with positive colitis control

Fig 1: Histopathological Studies in Preventive and Therapeutic Model

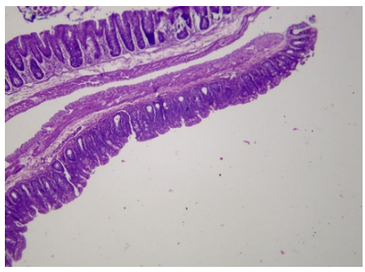
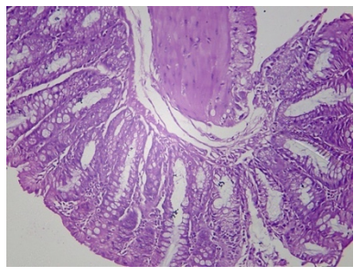
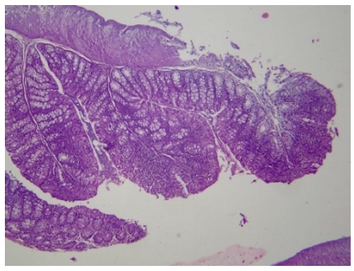
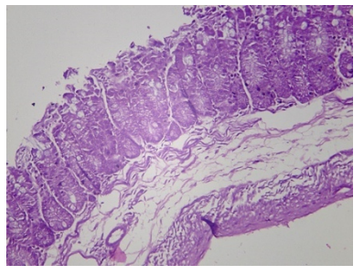
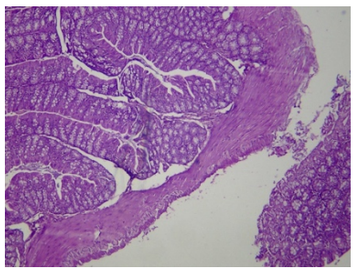
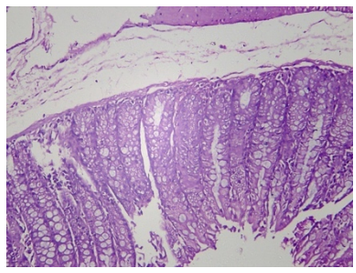
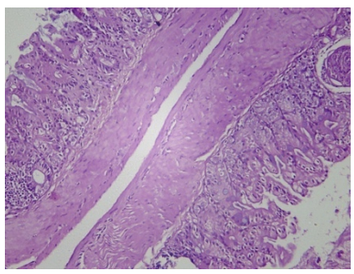
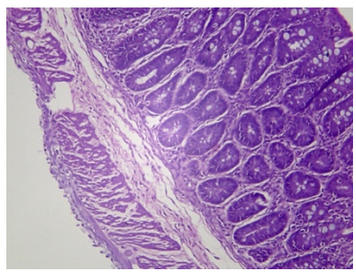
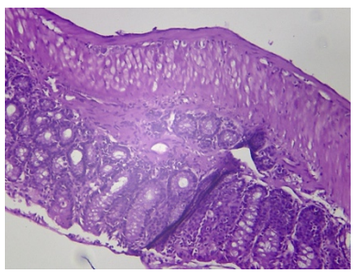
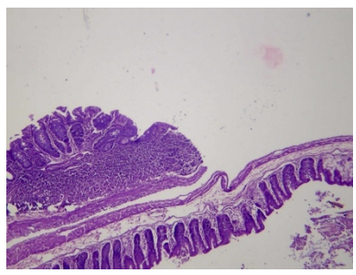
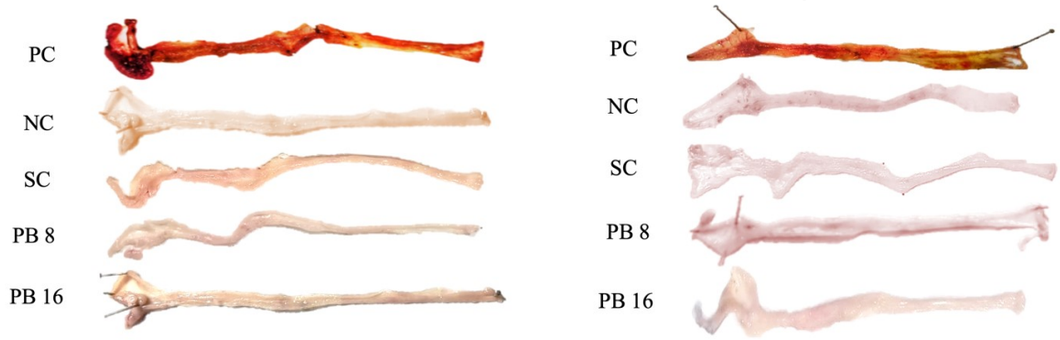
Preventive Model		Therapeutic Model	
Negative control The negative control tissue showed normal histological structure, including the mucosa (lamina epithelialis, lamina muscularis mucosa and lamina propria), the submucosa, the muscular coat (inner circular and outer longitudinal muscle fibres) and the serosa.			**Negative control** The negative control tissue showed normal histological structure, including the mucosa (lamina epithelialis, lamina muscularis mucosa and lamina propria), the submucosa, the muscular coat (inner circular and outer longitudinal muscle fibres) and the serosa.
Positive control The positive control tissue showed multifocal mucosal ulcerations accompanied with severe tissue damage in the entire mucosal layers, severe inflammatory reaction with congestion in mucosal and submucosal blood vessels was also pronounced.			**Positive control** The positive control tissue showed, massive necrosis and desquamative changes were found in the superficial and deep epithelial cell associated with leukocyte infiltration mainly lymphocytes and a few neutrophils. The colon glands found focally deformed with no secretory activity.
Standard control The Standard control tissue showed a healed ulcerative lesion with regeneration of the mucosal epithelium. In addition, there was moderate inflammatory reaction noticed.			**Standard control** The standard control tissue showed a healed ulcerative lesion with regeneration of the mucosal epithelium. In addition, there was moderate inflammatory reaction noticed.
Sample 8 days The Sample 8 days tissues showed regenerated epithelial cells, focal hyperplastic changes with hyperchromatic nuclei (regenerative signs). The mucosal proximity of the site of the healed ulcer was greatly infiltrated by inflammatory cells mostly lymphocytes and plasma cells.			**Sample 8 days** The Sample 8 days tissues showed, mild polymorphic infiltration, healthy mucosa can be seen. The inflammation extending through the muscularis mucosae and submucosa was observed.
Sample 16 days The Sample 16 days tissues showed regenerated epithelial cells, the submucosa markedly replaced by aggregated and/or follicular hyperplastic lymphoid cells.			**Sample 16 days** The Sample 16 days tissue showed, largely preserved mucosal lining with insignificant inflammation. Histopathological changes were significantly attenuated, as judged by epithelialization of the mucosa, reduction of edema and inflammatory cells recruitment.

Figure 2: MACROSCOPIC ALTERATIONS ON THE MICE COLON IN PREVENTIVE AND THERAPEUTIC MODEL



Positive Control (PC), Negative Control (NC), Standard Control (SC), *Piccha basti* Sample 8 days (PB 8), *Piccha basti* Sample 16 days (PB 16).

CONCLUSION

Piccha basti has shown significant protective action in the colon against experimental Ulcerative colitis in C57BL/6 mice. Histopathological and ELISA tests for anti – inflammatory markers revealed that *Piccha basti* has better results than the standard treatment using mesalamine enema against experimental Ulcerative colitis in C57BL/6 mice. Considering the results of the study, *Piccha basti* can be identified as a better treatment modality to industry standards and can be suggested for further clinical studies to evaluate the symptomatic efficacy.

Sample 8 days and sample 16 days did not show significant anti–oxidant action. However, the *basti dravyas* can be modified to include drugs that have proven anti-oxidant property to better suit the need for Ulcerative colitis.

A combination of preventive and therapeutic model of *Piccha basti* can be compared against a model with only therapeutic approach. If the hypothesis that the combination approach is more beneficial is proven, then it can help to develop combined protocol for management in the population which is vulnerable to Ulcerative colitis.

Another pattern of administration called *Karma basti*, wherein *Basti* is administered for a longer duration (30 days), can also be subjected to comparative study, given its superiority and wide use in clinical practice. Also, with an aim to prevent episodes of Ulcerative colitis, there poses a challenge that the periodicity and vulnerability of the disease can not be well predicted to intervene accordingly. Thus, the *Karma basti* pattern of longer duration can be proven to be a more comprehensive and effective approach.

As *Piccha basti* has shown significant overall improvement in Ulcerative colitis, similar efficacy could be expected in Crohn's disease as well. Based on the results of this study, studies to evaluate the efficacy of *Piccha basti* in Crohn's disease can also be carried out, owing to the similarities in the clinical presentation and underlying etiopathology between Ulcerative colitis and Crohn's disease.

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