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Research Article

Effect of *Alternanthera brasiliana* in experimentally induced inflammatory bowel disease in albino rats

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ABSTRACT

Background: Inflammatory bowel disease (IBD) is a chronic inflammatory condition affecting the gastrointestinal tract with limited therapeutic options. The present study aimed to investigate the effect of methanolic extract of *Alternanthera brasiliana* in acetic acid-induced inflammatory bowel disease (IBD) in albino rats.
Methods: A total of 36 animals were included in the study. Animals were divided into six groups (n = 6): group I - control (normal saline), group II-AA+ normal saline, group III-Sulfasalazine (360mg/kg)+AA, group IV A-methanolic extract of *Alternanthera brasiliana* (300 mg/kg), group IV B-methanolic extract of *Alternanthera brasiliana* (600 mg/kg)+AA, group IV C-methanolic extract of *Alternanthera brasiliana* (900 mg/kg)+AA. Group IV was divided into three subgroups, namely IVA, IV B and IV C, on the basis of different doses of methanolic extract of *Alternanthera brasiliana*. After completion of 7 days of treatment, rats were sacrificed under ether anesthesia for assessment of intestinal inflammation using parameters namely colon weight change, macroscopic and histopathological evaluation.
Results: There was a statistically significant decrease in colonic weight, macroscopic scores and microscopic scores in groups treated with methanolic extract of *Alternanthera brasiliana* at a dose of 600 mg/kg.
Conclusions: The present study indicates the efficacy of methanolic extract of *Alternanthera brasiliana* in acetic acid-induced IBD. The effects are more pronounced at a dose of 600 mg/kg than at 300 mg/kg and 900 mg/kg of methanolic extract of *Alternanthera brasiliana*.

Keywords: Inflammatory bowel disease, Methanolic extract of *Alternanthera brasiliana*, Colon weight change, Colon weight, Macroscopic evaluation, Histopathological evaluation

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic condition affecting the intestine. Ulcerative colitis (UC) and Crohn's disease (CD) are the two major types. The incidence of IBD, especially UC, is on the rise in Japan, South Korea, Singapore, northern India and Latin America, areas previously thought to have low incidence.[1]

Patients with CD and UC alternate between periods of active disease, which may require hospital admission, and periods of remission. The cause of IBD seems to include genetic, environmental, and immunologic components. Evidence suggests that IBD is triggered by an aberrant immune response to enteric flora, leading to intestinal inflammation.[2,3]

Pathophysiological changes in IBD are well established, among which cytokines like tumor necrosis factor α (TNF- α), interleukin-1 (IL-1) and interleukin-8 (IL-8) are secreted from macrophages. 4 TNF- α upregulates the adhesion molecules (E-selectin and ICAM-1) causing the adherence of neutrophils in endothelium and passage into the bowel wall. Activated neutrophils are attracted following release of IL-8, and undergo degranulation to release toxic proteases and reactive oxygen species, which are cytotoxic in nature and cause intestinal ulceration.[4] Frontline drugs that are currently used to treat IBD includes 5-aminosalicylic acid (sulfasalazine), corticosteroids (prednisolone), immunomodulatory drugs (azathioprine, mercaptopurine, methotrexate), IgG anti-TNFα antibody (infliximab) and antibiotics (metronidazole, ciprofloxacin).[5] These drugs have varying efficacy from patient to patient, and long-term use of these drugs can have harmful side effects. In view

of the devastating nature of IBD and the limited efficacy of the drugs used for its treatment, it would be very helpful to have other effective anti-inflammatory drugs.

Alternanthera Brasiliana Kuntz belonging to family Amaranthaceae is an herbaceous plant commonly known in ayurveda as Matsyaakshi, Matsyaakshika or Minaakshi, in Siddha medicine as Ponnonkanni, in Unani medicine as Machhechhi, in Tamil as Ponnonkanni.[6] It is known as Penicillina or Brazilian joy weed in Brazil, where it is employed as analgesic and anti-inflammatory agent.[7] Geographically, it is found throughout hotter regions of India, ascending to an altitude of 1,200 m in Himalayas; also cultivated as a pot-herb.[8] Pharmacological assays have proven anti-nociceptive activities in mice, anti-microbial effect and antiviral effects against herpes simplex.[9-11] Wound healing, antioxidant and angiogenic activity have also been demonstrated.[12] It is also proved to block human mitogen-induced lymphocyte proliferation.[13] There is an increased interest in plants as sources of new drugs because they are easily available, economical and can circumvent toxicities of modern medicine.[14]

Several studies have demonstrated the role of excessive inflammation and oxidative stress in the pathogenesis of UC.[15] Amelioration of LPO as well as free radicals scavenging would provide a protective therapy for UC. Barua CC et al, has proven *Alternanthera brasiliana* to possess antioxidant and anti-inflammatory activity, hence *Alternanthera brasiliana* would be expected to reduce inflammation and/or improve tissue healing following injury from UC.[10] Hence, the present study was undertaken with the objective to evaluate the anti-inflammatory effect of methanolic extract of *Alternanthera Brasiliana* in experimentally induced IBD in albino rats.

METHODS

The present study was conducted in the Department of Pharmacology, Gauhati Medical College, Guwahati, India. Adult wistar albino rats of either sex weighing between 150-250 gm were procured from the institute central animal house. The animals were housed in standard laboratory conditions at 25 °C and 12 hours light and dark cycle. Animals were given free access to rat chow diet and water ad libitum. Animals were familiarized to laboratory environment for seven days before conducting the experiment.

Drugs and chemicals needed for the study are sulfasalazine (Cadila), acetic acid (AA) and methanolic extract of *Alternanthera brasiliana* (MEAB).

Experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Gauhati Medical College, Guwahati (IAEC No: 351/CPCSEA/3/1/2001), India. The study was performed in accordance to the CPCSEA guidelines.

Dose and route of administration

- Sulfasalazine - 360mg/kg per oral (P.O) 16
- MEAB (P.O)
- Acetic acid (AA) - 1ml of 4% transrectally (T.R)

Methods

Total 36 animals were included in the study. Animals were divided into four groups I, II, III and IV. Group IV was subdivided into three subgroups A, B and C. Each group and subgroup consisted of six animals.

- Group I: Control group
- Group II : Acetic acid treated group
- Group III: Positive control group - Sulfasalazine (360mg/kg) + 4% AA
- Group IV: Test group (MEAB + AA)
- Group IV: Sub group A- MEAB (300 mg/kg) + 4% AA
- Group IV: Sub group B - MEAB (600 mg/kg) + 4% AA
- Group IV: Sub group C - MEAB (900 mg/kg) + 4% AA

Induction of colitis

IBD was induced according to the procedure described by MacPherson and Pfeiffer.[17] Briefly, rats were slightly anaesthetized with ether following 24 hour fast, a soft 6 F pediatric catheter lubricated with lignocaine jelly was inserted rectally into the colon through anus such that tip is 8 cm proximal to anus, approximately at the splenic flexure. Then 1ml 4% acetic acid was introduced into the colon and, after 30s of exposure, the fluid was withdrawn. As previously shown, intrarectal administration of 4% acetic acid produces colonic inflammation in rats similar to that in humans with ulcerative colitis.[18] The experimental animals were divided into mainly 5 groups.

Group I

(Normal saline treated group) in this group 1 ml of normal saline was delivered intrarectally to the rats after ether anesthesia as method described earlier.

Group II

(Acetic acid treated group) 1 ml of 4% acetic acid was delivered intrarectally to the rats after ether anesthesia as mentioned earlier to induce colitis.

Group III

(Sulfasalazine treated group) Rats received sulfasalazine 360mg/kg of rat body weight, orally daily by intra-gastric tube for 7 days. On 7th day, 1hr after sulfasalazine

administration rats was given 1ml of 4% acetic acid intrarectally after ether anesthesia.

Group IV

(MEAB treated group) Animals were divided into three subgroups on the basis of different doses of MEAB.

Group IV sub group A

MEAB in the dose of 300 mg/kg body weight of rat was given orally once daily by intra-gastric tube for 7 days. On the 7th day, 1 hour after MEAB administration rats was given 1ml of 4% acetic acid intrarectally after ether anesthesia.

Group IV sub group B

MEAB in the dose of 600 mg/kg body weight of rat was given orally once daily by intra-gastric tube for 7 days. On the 7th day, 1hr after MEAB administration rats was given 1ml of 4% acetic acid intrarectally after ether anesthesia.

Group IV sub group C

MEAB in the dose of 900 mg/kg body weight of rat was given orally once daily by intra-gastric tube for 7 days. On the 7th day, 1hr after MEAB administration rats was given 1ml of 4% acetic acid intrarectally after ether anesthesia.

Assessment of colonic damage

The parameters assessed were colon weight change, macroscopic evaluation and histopathological evaluation and given macroscopic and microscopic score. The assessment was carried out in the Department of Pathology, Gauhati Medical College and Hospital, India.

Colon weight change

The weight of damaged colon tissue is considered an indicator of the severity and extent of inflammatory response, where an increase in colonic weight represents inflammation and a decrease in colonic weight following treatment indicates anti-inflammatory activity.

Macroscopic evaluation

Twenty-four hours following induction of colitis, animals were euthanized by ether and 10cm of distal colon was removed from surrounding tissues, opened longitudinally along its mesenteric border, rinsed, and processed for histology. After washing the mucosa with saline solution, gross examination of mucosal injury was done using the grading scale of Morris et al.[19]

- Score 0 - No damage
- Score 1 - Localized hyperemia but no ulcers
- Score 2 - Linear ulcers with no significant inflammation
- Score 3 - Linear ulcer with inflammation at one site
- Score 4 - Two or more sites of ulceration and inflammation
- Score 5 - Two or more sites of ulceration and inflammation or one major site of Inflammation and ulceration extending >1 cm along the length of the colon.

Histopathological evaluation

Additional samples were fixed in 10% formalin in phosphate buffered saline, embedded in paraffin, and cut into 4µm sections. Paraffin sections were deparaffinized with xylene, hydrated, and stained with hematoxylin and eosin. The degree of inflammation was graded semi quantitatively from 0 to 11 as the sum of: 20

- Loss of mucosal architecture (score-3)
- Cellular infiltration (score 0-3)
- Muscle thickening (score 0-3),
- Crypt abscess formation (score 0-1)
- Goblet cell depletion (score 0-1)

Statistical analysis

All the data were entered in to data base program. Data were expressed as mean±SEM . Results were analyzed by one way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. P-value <0.05 was considered as statistically significant.

RESULTS

Colon weight changes

Mean colonic weight of group I was 4.38 ± 0.18 which is statistically significant (p <0.01) when compared to group II with a mean colonic weight of 9.67±0.22. (Table 1, Figure 1A) Mean colonic weight of group III was 4.74±0.08 which was statistically significant (p <0.01) when compared with group II. In animals pretreated with MEAB at a dose of 600 mg/kg (group IV B), mean colonic weight was 5.48±0.20 which was statistically significant (p <0.05), whereas in animals pretreated with MEAB at a dose of 300 mg/kg (group IV A) and 900 mg/k (group IV C) mean colonic weight were 8.91±0.17 and 7.11±0.10 which when compared to group II were statistically not significant.

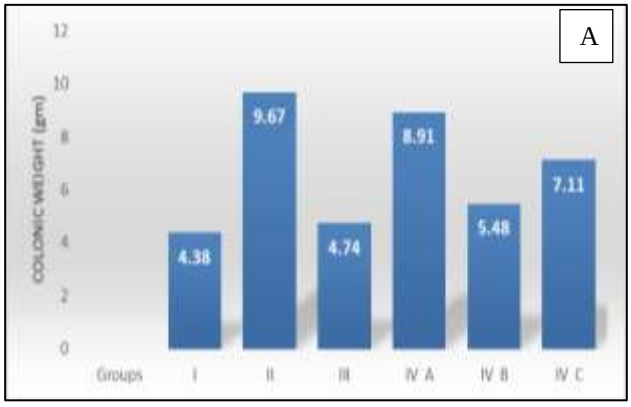
Table 1: Parameters for assessment of colonic inflammation.

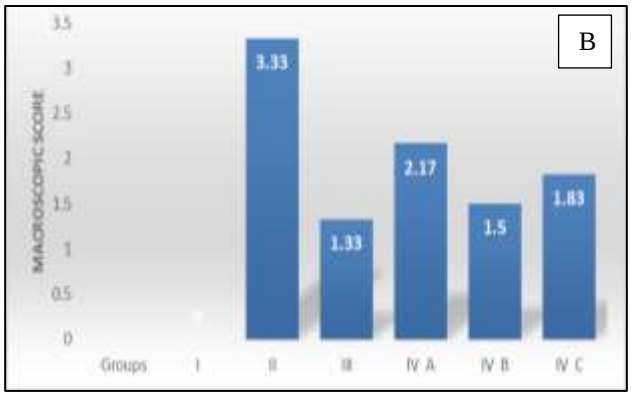
Groups	Treatment	Colon weight change (gm)	Macroscopic score	Microscopic score
I	Normal saline	4.38±0.18*	0.0*	0.0*
II	AA treated	9.67±0.22	3.33±0.52	3.83±0.41
III	Sulfasalazine + AA	4.74±0.08*	1.33±0.52*	1.17±0.41*
IV A	MEAB (300 mg/kg) + AA	8.91±0.17	2.17±0.75	2.50±0.55
IV B	MEAB (600 mg/kg) + AA	5.48±0.20*	1.50±0.84*	1.50±0.55*
IV C	MEAB (900 mg/kg) + AA	7.11±0.10	1.83±0.75	2.00±0.63

Data are expressed as mean ± SEM (n=6) and analyzed using one way ANOVA followed by Dunnett's t test. The statistical significance was considered as significant if * $p < 0.05$ when compared with acetic acid group.

Macroscopic evaluation

Mean values of macroscopic score of group I was 0.0±0.0 which is statistically significant ($p < 0.01$) when to compared group II with a score of 3.33±0.52. (Table 1, Figure 1B) Mean macroscopic score of group III was 1.33±0.52 which is statistically significant ($p < 0.01$) when compared with group II. In animals pretreated with MEAB at a dose of 600 mg/kg (group IV B), mean macroscopic score was 1.50±0.84 which was statistically significant ($p < 0.05$), whereas in animals pretreated with MEAB at a dose of 300 mg/kg (group IV A) and 900 mg/k (group IV C) mean macroscopic score were 2.17 ± 0.75and 1.83 ± 0.75which when compared to group II were statistically not significant.





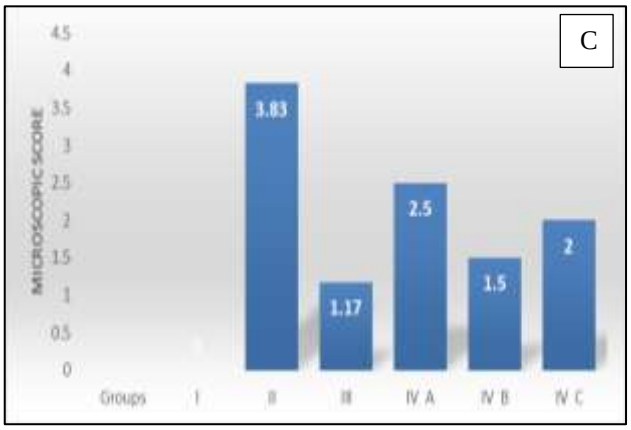
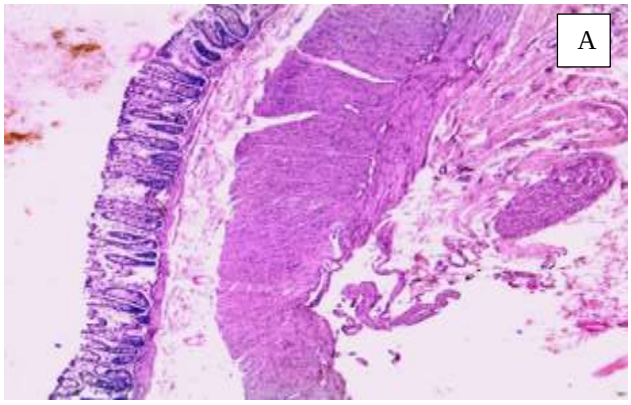


Figure 1: Effect of methanolic extract of *Alternanthera Brasiliana* in experimentally induced IBD in albino rats. A) Colon weight change (gm); B) Macroscopic evaluation; C) Histopathological evaluation.

Histopathological evaluation

Mean microscopic score of group I was 0.00 ± 0.00 which is statistically significant ($p < 0.01$) when compared to group II with a score of 3.83±0.41. (Table 1, Figure 1C) Mean microscopic score of group III was 1.17 ± 0.41which is statistically significant ($p < 0.01$) when compared with group II.



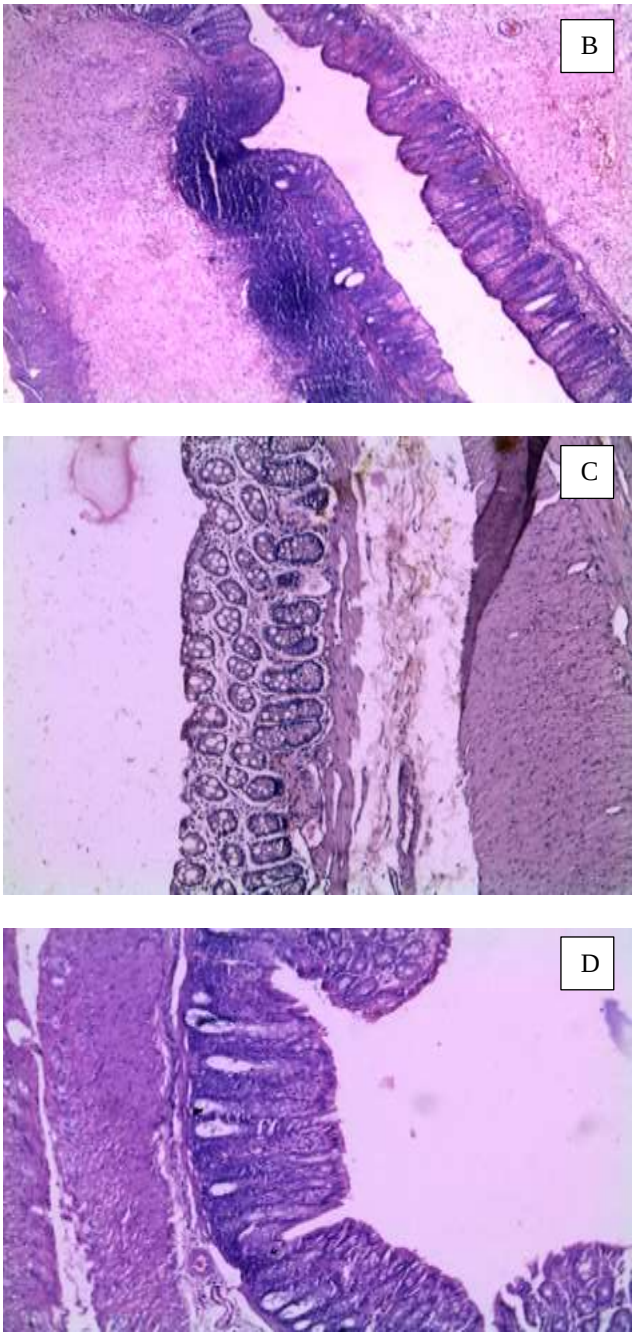


Figure 1: Histopathological sections of colons from rats stained with H&E. Colonic microscopic image of A) Normal rat colon from Control group I with intact mucosal layer and epithelial; B) AA treated (group II) rat colon with diffused active colitis, extensive damage including edema of mucosa and submucosa and chronic inflammatory cells infiltrate with widely ulcerating mucosa, and haemorrhages; C) Sulfasalazine treated (group III) colon with reduced active colitis, reduced mucosal ulcer and minimal inflammatory cell infiltrates; D) MEAB 600 mg/kg(group IV B) treated colon showing reparative epithelial changes and ulcer healing with lymphoid follicle in colon.

In animals pretreated with MEAB at a dose of 600 mg/kg (group IV B), mean microscopic score was 1.50±0.55 which was statistically significant ($p < 0.05$), whereas in animals pretreated with MEAB at a dose of 300 mg/kg (group IV A) and 900 mg/k (group IV C) mean microscopic score were 2.50±0.55 and 2.00±0.63 which when compared to group II were statistically not significant (Figure 2).

Thus from the above results, it is seen that MEAB at a dose of 600 mg/kg reduced colon weight and decreased macroscopic and microscopic score significantly

DISCUSSION

IBD is a chronic intestinal condition with dysregulated mucosal immune function. Two major types are UC and CD. UC is a mucosal disease that usually involves the rectum and extends proximally to involve the colon manifesting with diarrhea, rectal bleeding, tenesmus, passage of mucus and crampy abdominal pain.[1]

Alternanthera brasiliana Kuntze (Amaranthaceae) is an herbaceous plant used in inflammation, cough and diarrhea in Brazilian popular medicine.[7] The plant methanolic extracts has demonstrated analgesic effects in mice, antimicrobial effect and also anti-herpes-simplex-virus activity.[9-11] Aqueous or ethanolic extracts of *A brasiliana* were able to block human mitogen-induced lymphocyte proliferation.[13] The methanolic extract has demonstrated wound healing, antioxidant and angiogenic activity.[12] Phytochemical studies have shown the presence of various chemical constituents such as flavonoids like kaempferol, quercetin, esters like stigmasterol, β-sitosterol 8 and various elements.[21]

Histological features in acetic acid induced colitis model are comparable to human ulcerative colitis. Acetic acid brings about inflammation, necrosis, edema, neutrophil infiltration and ulceration of the mucosal and submucosal layers of distal colon. Massive epithelial damage occurs due to intracellular acidification as a result of protons released from acetic acid into intracellular space.[22] A key factor involved in the pathogenesis of IBD is oxidative stress.[23] Intestinal mucosal damage that occurs in IBD, is related to both increased free radical production and a low concentration of endogenous antioxidant defense.[24]

The present study was undertaken to assess the anti-inflammatory effect of MEAB in experimentally induced IBD in albino rats. The parameters assessed were colon weight change, macroscopic and histopathological evaluation and given macroscopic and microscopic score. Increased colon weight, macroscopic and microscopic scores indicated inflammatory activity whereas reduced colon weight and scores implied anti-inflammatory activity. The results of the present study demonstrated that MEAB reduced mean colonic weight, macroscopic and microscopic scores (statistically significant) at dose of 600 mg/kg. MEAB at doses of 300 mg/kg and 900 mg/kg

also reduced colonic weight, macroscopic and microscopic scores, but were statistically not significant. The anti-inflammatory activity of MEAB was less when compared to Sulfasalazine. This suggests MEAB to possess anti-inflammatory activity. Further assessment of *Alternanthera brasiliana* with changes in the dosage, solvent extracts and other chronic inflammatory models, will throw more light on its chronic anti-inflammatory activity. The exact mechanism responsible protective activity of MEAB remains unclear, which may be mediated by multiple mechanisms. Further studies are needed to assess the exact mechanism and characterize the active principles responsible for ulcerative colitis protective activity.

CONCLUSION

A large number of studies on *Alternanthera brasiliana* have showed anti-nociceptive effects, antimicrobial effect, anti-herpes-simplex-virus activity, anti-lymphocyte proliferator activity, wound healing, anti-oxidant and angiogenic activity. In the present study MEAB has showed promising results in Acetic acid induced colitis model of IBD. These studies are valuable for identifying lead compounds for anti-inflammatory drugs, keeping in mind the side effects of modern therapy for UC. Further human studies are needed to prove the safety and efficacy of long term administration of MEAB as potential anti-inflammatory agent in routine clinical practice.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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