STUDY OF THE EFFECT OF THE EXTRACT OF THE LEAVES OF 
ALTERNANTHERA BRASILIANA IN EXPERIMENTALLY INDUCED 
INFLAMMATORY BOWEL DISEASE IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objectives: To evaluate the effect of the ethanolic extract of leaves of Alternanthera brasiliana (EEAB) in different doses in 2,4,6-Trinitrobenzenesulfonic acid (TNBS) induced inflammatory bowel disease in albino rats. Methods: The study was done on healthy albino rats in the Department of Pharmacology of Gauhati Medical College. They were housed in standard laboratory conditions. There were 6 groups each consisting of 6 animals. The groups were Group I (Normal Control), Group II (Disease Control), Group III (Standard), Group IV (EEAB 200 mg/kg), Group V (EEAB 400 mg/kg) and Group VI (EEAB 600 mg/kg). Experimental colitis was induced in all the groups except in Group I on 1st day. The animals were treated with sulfasalazine and different doses of the extract for the next 14 days. The animals were sacrificed on 15th day under anaesthesia. The colonic damage was assessed by observing the colon weight change, macroscopic and histopathological examination. Results: The data was analyzed using ANOVA and Dunnett’s test was used as post ANOVA analysis using Group II as control column. The results with p<0.05 are considered to be significant. Conclusions: The EEAB was found to have significant anti-inflammatory activity in experimentally induced colitis as shown by the decrease in the colon weight, macroscopic score and histopathological score.

KEYWORDS: Alternanthera brasiliana, Experimentally induced colitis, TNBS induced colitis, Anti-inflammatory activity.
INTRODUCTION

Inflammatory bowel disease (IBD) is an immune-mediated chronic intestinal disease.[1] The two main disease categories the term covers are Crohn’s disease (CD) and Ulcerative colitis (UC), with both overlapping and distinct clinical and pathological features.[2]

IBD is a very disabling disease due to the fatigue associated with the inflammatory symptoms and due to the chronic pain suffered by patients. IBD affects quality of life but not life span: the mortality rate of patients is not different from the normal population.

Medical therapy for IBD is problematic. Because no unique abnormality has been identified, current therapy for IBD seeks to dampen the generalized inflammatory response; however, no agent can reliably accomplish this and the response of an individual patient to a given medicine may be limited and unpredictable. For many years glucocorticoids, sulfasalazine and 5-aminosalicylic acid (5-ASA) were the mainstays of pharmacotherapy for IBD. More recently azathioprine and cyclosporine (CSA), have been adapted for IBD therapy. However, the use of these drugs is accompanied by a certain number of side effects, with some of them being quite severe.[3] Therefore new therapeutic approaches are needed to treat IBD more efficiently.

Several cytokines including tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1β have been shown to amplify and perpetuate tissue damage in IBD. Furthermore, chemokines are also upregulated, thus providing a continuous signal for the influx of leucocytes.[4]

*Alternanthera brasiliana* (L.) O kuntze (Amaranthaceae) is evergreen, perennial herb native to tropical and sub-tropical regions of Australia and South America.[5] It has been used for its anti-microbial, analgesic and anti-inflammatory properties.[6] The preliminary qualitative phytochemical screening of hexane, chloroform and methanol extracts indicated the presence of alkaloids, phenolic groups, flavonoids, saponins, tannins, phytosterols and carbohydrates.[7]

The results of previous studies on different properties of *Alternanthera brasiliana* are very encouraging but very few studies are available regarding its effect in inflammatory bowel disease. As this plant has been mentioned in the literature and traditional medicine being used in inflammation and as its use in IBD is not properly documented, so this study has been taken up to evaluate its anti-inflammatory property in experimental models of IBD. It aims to
evaluate the anti inflammatory effect of different doses of EEAB in TNBS induced IBD in rats. Hope this humble effort will throw some light in this field of research.

MATERIALS AND METHODS
The study was conducted in the Department of Pharmacology, Gauhati Medical College, Guwahati.

Ethical review
The protocol was submitted to the Institutional Animal Ethics Committee of Gauhati Medical College and Hospital, Guwahati bearing CPCSEA Registration No. 351, 3/1/2001. It was approved by the Committee bearing approval no MC/05/2015/17 and the CPCSEA guidelines were adhered during the study.

Drugs and chemicals used in the study
1) Ethanolic extract of Alternanthera brasiliana. (EEAB)
2) Sulfasalazine procured from Wallace Pharmaceuticals Ltd.
3) Tri Nitro Benzene Sulphonic acid (Picrylsulfonic acid) obtained from Sigma Aldrich (TNBSA, Catalog No P2297-10ML)
4) Vehicle: Normal Saline (0.9% NaCl)

Extraction of plant material It was done using Soxhlet apparatus.

Experimental animals used in the study
The study was carried out in healthy albino rats of either sex weighing 150-200 gm. They were fed rat chaws diet and water ad libitum during the experiment. Animals were maintained under controlled condition with 12 hour light and 12 hour dark cycles at a temperature of 24 ± 1°C. Before conducting the experiment all the animals were acclimatized to laboratory condition for 7 days.

Experimental Design
The study comprised of 6 groups each consisting of 6 animals. The groups are as follows:

**Group I:** Normal Saline at dose of 10 ml/kg per oral

**Group II:** TNBS per rectally on 1\textsuperscript{st} day only

**Group III:** TNBS(1\textsuperscript{st} day) + Sulfasalazine (360mg/kg p.o)\textsuperscript{8} for the next 14 days

**Group IV:** TNBS (1\textsuperscript{st} day) + EEAB (200 mg/kg p.o) for the next 14 days

**Group V:** TNBS (1\textsuperscript{st} day) + EEAB (400mg/kg p.o) for the next 14 days
Group VI: TNBS (1\textsuperscript{st} day) + EEAB (600mg/kg p.o) for the next 14 days

**Induction of Colitis**

Experimentally colitis was induced by a single intra-colonic application of TNBS (20 mg dissolved in 0.25 ml of 35% ethanol).\[^9\] Prior to induction the animals were fasted for a period of 12 hours. Rat is placed under light ether anaesthesia and a flexible polyethylene tube lubricated with lignocaine jelly is inserted per rectally into the colon through the anal verge such that the tip is 8cm proximal to anus, approximately at splenic flexure. TNBS is dissolved in 35% ethanol (v/v) and instilled into the lumen of the colon through the catheter. The rats were maintained in head down position for 1 minute to prevent leakage and for even distribution of TNBS.\[^10, 11\] The total volume is expelled with additional air and the catheter is removed. Rats were observed for a period of 14 days. On 15\textsuperscript{th} day animals were sacrificed under anaesthesia for demonstration of colitis.

**Colon weight**

Colon weight was calculated in all the groups after the animals were sacrificed on the 15\textsuperscript{th} day.

**Assesment of Colonic Damage**

All the animals were sacrificed on the 15\textsuperscript{th} day under anaesthesia with Ketamine. The abdomen was opened and the colon was exposed. 10cm of the distal colon was removed from the surrounding tissues and it was opened longitudinally along its mesenteric border. After washing the mucosa with saline solution, mucosal injury was assessed macroscopically using the grading scale of Morris \textit{et al}.\[^12\]

- 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 >1cm along the length of the colon.
**Histopathological examination**

The Histopathological examinations of all the samples were done in the Department of Pathology, Gauhati Medical College and Hospital. The histopathological score was assessed following modified model of Wei *et al*[^13] as follows:

1. The infiltration of acute inflammatory cells:
   - 0-no, 1-mild increasing, 2-severe increasing;
2. The infiltration of chronic inflammatory cells:
   - 0-no, 1-mild increasing, 2-severe increasing;
3. The deposition of fibrin protein:
   - 0-negative, 1-positive;
4. The submucosa edema:
   - 0-no, 1-patchy-like, 2 fusion-like
5. The epithelium necrosis:
   - 0-no, 1-limiting, 2-widening
6. The epithelium ulcer:
   - 0-negative, 1-positive.

**RESULTS AND OBSERVATIONS**

The assessment of colonic damage was done by observing the colon weight change, macroscopic and histopathological examination. The data was analyzed and one way analysis of variance (ANOVA) was carried out. Dunnett’s test was used as post ANOVA analysis using Disease Control (Group II) as control column. The \( p \) values less than 0.05 are considered to be significant. The statistical analysis was carried out using Graph pad prism 5.01 software.

**Colon Weight**

The results obtained for this parameter are summarized in Table 1. The values of colon weight in different groups are expressed in gm.

**Table 1: Colon Weight (gm)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Control</td>
<td>4.17±0.11*</td>
</tr>
<tr>
<td>Group II</td>
<td>Disease Control</td>
<td>10.05±0.21</td>
</tr>
<tr>
<td>Group III</td>
<td>Standard</td>
<td>4.63±0.12*</td>
</tr>
<tr>
<td>Group IV</td>
<td>EEAB 200mg/kg</td>
<td>7.42±0.14*</td>
</tr>
<tr>
<td>Group V</td>
<td>EEAB 400mg/kg</td>
<td>6.25±0.09*</td>
</tr>
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</table>
The results of one way ANOVA for colon weight is statistically significant (p<0.05). Values are expressed as Mean ± SEM (n=6). One Way ANOVA followed by Dunnett’s multiple comparison tests is done. * p<0.05 when compared to the Group II (Disease Control).

MACROSCOPIC SCORING
The results for this parameter are summarized in Table 2.

Table 2: Macroscopic Scoring

<table>
<thead>
<tr>
<th>Groups</th>
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<tbody>
<tr>
<td>Group I</td>
<td>Normal Control</td>
<td>0.33 ± 0.21*</td>
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<tr>
<td>Group II</td>
<td>Disease Control</td>
<td>4.83 ± 0.17</td>
</tr>
<tr>
<td>Group III</td>
<td>Standard</td>
<td>1.67 ± 0.21*</td>
</tr>
<tr>
<td>Group IV</td>
<td>EEAB 200mg/kg</td>
<td>3.67 ± 0.21*</td>
</tr>
<tr>
<td>Group V</td>
<td>EEAB 400mg/kg</td>
<td>2.83 ± 0.17*</td>
</tr>
<tr>
<td>Group VI</td>
<td>EEAB 600 mg/kg</td>
<td>2.17 ± 0.17*</td>
</tr>
</tbody>
</table>

One way ANOVA  
F 68.54

df 5,30

p <0.05

Comments
The results of one way ANOVA is statistically significant (p<0.05). Values are expressed as Mean ± SEM (n=6). One Way ANOVA followed by Dunnett’s multiple comparison tests is done. * p<0.05 when compared to Group II (Disease Control).

HISTOLOGICAL EXAMINATION
The results obtained for this parameter are summarized in Table 3 & 4.

Table 3 shows Mean ± SEM values of infiltration of acute inflammatory cells, infiltration of chronic inflammatory cells, deposition of fibrin protein, submucosal oedema, epithelial ulcer and epithelial necrosis in colon tissue of each group.
Table 4 shows the Mean ± SEM values for the total histopathological score obtained in each group. The total histopathological score was calculated by adding up the scores obtained for each individual parameter for all the groups and the Mean ± SEM values were calculated thereafter for each group.

Table 3: Histopathological Score

<table>
<thead>
<tr>
<th>Groups</th>
<th>Infiltration of acute inflammatory cells</th>
<th>Infiltration of chronic inflammatory cells</th>
<th>Fibrin deposition</th>
<th>Submucosal oedema</th>
<th>Epithelium ulcer</th>
<th>Epithelium necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0±0*</td>
<td>0±0*</td>
<td>0±0*</td>
<td>0±0*</td>
<td>0±0*</td>
<td>0±0*</td>
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<tr>
<td>Group II</td>
<td>2±0</td>
<td>1.5±0.22</td>
<td>1±0</td>
<td>1.67±0.21</td>
<td>1±0</td>
<td>2±0</td>
</tr>
<tr>
<td>Group III</td>
<td>0.33±0.21*</td>
<td>0.17±0.17*</td>
<td>0±0*</td>
<td>0.33±0.21*</td>
<td>0±0*</td>
<td>0.33±0.21*</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.83±0.17*</td>
<td>0.67±0.21*</td>
<td>0.33±0.21*</td>
<td>0.83±0.17*</td>
<td>0.33±0.21*</td>
<td>0.83±0.17*</td>
</tr>
<tr>
<td>Group V</td>
<td>0.67±0.21*</td>
<td>0.5±0.22*</td>
<td>0.17±0.17*</td>
<td>0.67±0.21*</td>
<td>0.17±0.17*</td>
<td>0.67±0.21*</td>
</tr>
<tr>
<td>Group VI</td>
<td>0.5±0.22*</td>
<td>0.33±0.21*</td>
<td>0±0*</td>
<td>0.5±0.22*</td>
<td>0±0*</td>
<td>0.5±0.22*</td>
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ONE WAY ANOVA

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<th></th>
<th>F</th>
<th>df</th>
<th>p</th>
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<tr>
<td></td>
<td>17.07</td>
<td>5.30</td>
<td>&lt;0.05</td>
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Table 4: Total Histolopathological Score

<table>
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<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Control</td>
<td>0 ±0*</td>
</tr>
<tr>
<td>Group II</td>
<td>Disease Control</td>
<td>9.17±0.40</td>
</tr>
<tr>
<td>Group III</td>
<td>Standard</td>
<td>1.17±0.60*</td>
</tr>
<tr>
<td>Group IV</td>
<td>EEAB 200mg/kg</td>
<td>3.83±0.65*</td>
</tr>
<tr>
<td>Group V</td>
<td>EEAB 400mg/kg</td>
<td>2.83±0.65*</td>
</tr>
<tr>
<td>Group VI</td>
<td>EEAB 600mg/kg</td>
<td>1.83±0.48*</td>
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ONE WAY ANOVA

<table>
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<th></th>
<th>F</th>
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<th>p</th>
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<tbody>
<tr>
<td></td>
<td>39.13</td>
<td>5.30</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Comments

The results of one way ANOVA are statistically significant.

Values are expressed as Mean ± SEM, (n=6)

One Way ANOVA followed by Dunnett’s multiple comparison tests is done.

* p<0.05 when compared to the Group II (Disease Control)
One Way ANOVA followed by Dunnett’s multiple comparison tests is done. 
* p<0.05 when compared to the Group II (Disease Control).

**Photographs of Gross and Histological Sections**

**Group I – Normal Control**

**Group II- Disease Control**

**Group III- Standard**
Group IV - EEAB 200mg/kg

Group V - EEAB 400mg/kg

Group VI - EEAB 600mg/kg
DISCUSSION

TNBS is diluted in ethanol which disrupts the mucosal barrier.¹¹ TNBS dissolved in ethanol results in acute inflammation with ulcers in rat and mouse.¹² It has been shown that it can elicit immunologic responses and induce generation of IBD.¹³,¹⁴

Various phytochemicals like alkaloids, phenolic compounds, tannins and flavonoids are present in EEAB. Kaempferol and quercetin derivatives are the main flavonoids found in the plant Alternanthera brasiliana. The anti-inflammatory effects observed in vivo can be attributed to the effects of these flavonoids on T-cell function, thereby accounting for the medicinal properties of Alternanthera brasiliana.¹⁵ Kaempferol and quercetin has shown anti inflammatory property in invitro models of inflammation.¹⁶,¹⁷

Flavonoids also have mast cell stabilizing property.¹⁸,¹⁹ Mast cell degranulation causes mucus secretion, mucosal edema, increased gut permeability and release of various inflammatory mediators which may be responsible for some of the signs and symptoms of IBD.²⁰ So it might be one of the mechanisms due to which EEAB exerts its protective effect in IBD.

Many studies have revealed that an increase of oxidative stress and iNOS activity was a notable feature of IBD, which resulted in a pathological cascade of free radical reactions and further yielding more oxidative free radicals. Failures of the endogenous antioxidant defence mechanisms promote formation of excessive free radicals and consequent tissue damage.²¹ Studies carried out by Samudrala PK et al²² and Enechi OC et al²³ demonstrates the antioxidant property of Alternanthera brasiliana.

The effects of the extract of Alternanthera Brasiliana in this study may be related to these properties. The presence of flavonoids which apart from anti inflammatory activity have also been shown to prevent mast cell degranulation. Intercepting this further prevents the release of inflammatory mediators. By preventing the tissue damage due to the free radicals thus enhancing the endogenous antioxidant defense mechanism might be another added factor for its anti inflammatory activity in this study.

CONCLUSION

The EEAB significantly reversed the rise in the colon weight, decreased the mean macroscopic score and the total histopathological score in colon tissue significantly. The
effect of EEAB on all the above mentioned parameters were in a dose dependent manner i.e.
the highest dose of EEAB improved the IBD maximally. However, this beneficial effect of
EEAB on IBD was less effective than the standard drug, Sulfasalazine.

ACKNOWLEDGEMENTS
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their immense help while carrying out the study.

REFERENCES
1. Friedman S, Blumberg RF. Inflammatory Bowel Disease. In: Kasper DL, Fauci AS,
   Hauser SL, Longo DL, Jameson JL, Loscalzo J. Harrison’s Principles of Internal
2. Inflammatory Bowel Disease: A Global Perspective. World Gastroenterology
3. Wallace JL, Sharkey KA. Pharmacotherapy of Inflammatory bowel disease. In:
   Bruton
   LL, Lazo JS, Parker KL. Goodman and Gillman’s The Pharmacological Basis of the
4. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice
   deficient for multidrug resistant genes, mdr 1a, spontaneously develop colitis. J
   Immunmol 1998; 161(10): 5733-44.
5. Barua CC, Begum SA, Barua AG, Borah RS, Lahkar M. Anxiolytic and anticonvulsant
   activity of methanol extract of leaves of Alternanthera brasiliana (L.) Kuntze
   Antimicrobial Activity of Alternanthera brasiliana Kuntze (Amaranthaceae): a
7. Kanan M, Chandran RP, Manju S. Preliminary phytochemical and antibacterial studies on
   leaf extracts of Alternanthera brasiliana (L.) kuntze. International Journal of Pharmacy
   honey and sulfasalzine in combination to promote antioxidant defense system in
   583-90.
