Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: http://dx.doi.org/10.18203/2319-2003.ijbcp20150383

Research Article

Evaluation of anti-inflammatory activity of *Oxalis corniculata* in experimentally induced inflammatory bowel disease in rats

Arijit Dutta*, Chimi Handique, Mangala Lahkar

Department of Pharmacology, Gauhati Medical College and Hospital, Guwahati, Assam, India

Received: 14 June 2015
Accepted: 03 July 2015

***Correspondence to:**
Dr. Arijit Dutta,
Email: dutta_ab@yahoo.in

ABSTRACT

Background: *Oxalis corniculata* is traditionally well-known for its versatile uses. The present study was carried out to evaluate the anti-inflammatory activity of the ethanolic extract of *O. corniculata* (EEOC) leaves in experimentally induced inflammatory bowel disease in rats.
Methods: Rats were treated with the extract for 7 days following which acetic acid was used to induce colitis. Animals were euthanized, 24 hrs after induction of colitis and colon was removed and assessed for macroscopic injury, as well as also processed for histopathological examination. Sulfasalazine 360 mg/kg was used as the standard drug. The extract was used in 200 mg/kg, 300 mg/kg and 400 mg/kg doses.
Results: At all the three doses, the EEOC showed significant ($p<0.01$) anti-inflammatory activity in experimental models.
Conclusion: Results obtained in this study substantiate the anti-inflammatory effect of EEOC leaves.

Keywords: Anti-inflammatory, *Oxalis corniculata*, Inflammatory bowel disease

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine. Crohn's disease (CD) and ulcerative colitis (UC) are the principal types of IBD. The causes of both CD and UC remain unknown.[1] According to the currently accepted hypothesis, UC and CD result from a dysregulated response of the mucosal immune system toward intraluminal antigens of bacterial origin in genetically predisposed persons.[2-4] The incidence of IBD, especially UC, is rising in Japan, South Korea, Singapore, Northern India, and Latin America, areas previously thought to have low incidence.[5] The peak age of onset for IBD is 15-30 years old, although it may occur at any age. About 10% of cases occur in individuals younger than 18 years. UC is slightly more common in males; whereas CD is marginally more frequent in women.[6] UC is a mucosal disease that usually involves the rectum and extends proximally to involve all or part of the colon. CD usually presents as acute or chronic bowel inflammation. The site of disease influences the clinical manifestations. CD can affect any part of the gastrointestinal tract from mouth to anus. Unlike UC, which almost always involves the rectum, the rectum is often spared in CD.[7] Current drug treatment is aimed to induce and then maintain the patient in remission and ameliorate the disease's secondary effects rather than modify or reverse the underlying pathogenic mechanism. However, the management of IBD with the use of the conventional treatment is expensive and also associated with a number of side effects.[8] There is growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low-cost. Even the WHO approves the use of plant drugs for different diseases including diabetes.[9] The genus *Oxalis* belongs to the family Oxalidaceae with about 500 species, distributed in America, Africa, Europe and Asia.[10] *Oxalis corniculata* Linn., a sub-tropical plant being native of India, are commonly known as creeping wood sorrel. It is a delicate-appearing, low-growing, herbaceous plant and abundantly distributed in damp shady places, roadsides, plantations, lawns, nearly all

regions throughout the warmer parts of India, especially in the Himalayas up to 8000 feet - cosmopolitan.[11] Traditionally, the plant is well-known for its versatile medicinal uses like treatment for stomach ache, decoction of roots is useful for worms, giddiness, diarrhea, and dysentery.[12] Leaves are well-masticated and the juice is kept in mouth for some time to get relief from apthae.[13] The raw fresh leaves are crushed and directly applied on skin to treat eczema.[14]

The present study was carried out to evaluate the anti-inflammatory activity of the ethanolic extract of the *O. corniculata* (EEOC) leaves in experimentally induced bowel disease in rats.

METHODS

Experimental animals

The study was carried out in healthy rats of either sex weighing between 200 to 250 g and around 9 to 12 weeks age. The animals were fed on rat chaws diet and water *ad libitum* during the study. They were housed in plastic cages at a controlled temperature of 24±1°C and 12 hrs light and dark cycle and were acclimatized to laboratory condition for 7 days before the study was conducted. The study was approved by the Institutional Animal Ethics Committee of Gauhati Medical College and Hospital. CPCSEA guidelines were adhered during the experiment.

Plant materials

The leaves of *O. corniculata* were collected from in and around Guwahati. Authentication of the plant was done in the Department of Botany, Gauhati University, and a voucher specimen was preserved for further reference. The whole plant was thoroughly washed, shade-dried and then chopped to a coarse powder using a mixer grinder. Powder (200 g) was tightly packed in soxhlet apparatus and extracted employing ethanol as a solvent for 5 days at a temperature of 40-60°C using a heating mantle. The extract was filtered using Whatman filter paper No.1 and the filtrate was evaporated on a water bath until it gets concentrated. The jelly like extract of the leaves was collected in a petridish. A final yield of 40.5 g was obtained. The percentage yield of *O. corniculata* was 20.25% (w/w) with respect to the original dried powder. The extract was stored in a refrigerator at 4°C in labeled airtight containers for further use.

Drugs and chemicals

- Sulfasalazine 360 mg/kg
- Acetic acid 4%
- EEOC in a dose of 200, 300 and 400 mg/kg

Acute toxicity study

An acute toxicity study was done according to OECD 425 Guidelines. The animals were found to be alive at 2000 mg/kg per oral feeding of the EEOC.

Methodology

Induction of colitis

Prior to induction of colitis the different groups were treated with the respective drugs and extract for 7 days. Colitis was induced according to the procedure described by MacPherson and Pfieffer.[15] On the 7th day, rats were anesthetized with ether following 24 hrs fast, a soft pediatric catheter was lubricated with lignocaine jelly and was inserted rectally into the colon through anus such that the tip is 8 cm proximal to anus, approximately at splenic flexure. Then 1 ml of acetic acid was introduced into the colon, and after 30 sec of exposure the fluid is withdrawn. Administration of 4% acetic acid produces intracolonic inflammation in rats that resemble many histological characteristics of human UC.[16]

Experimental design

The animals were divided into following groups and each group containing six animals:

Group I: Control group received normal saline at a dose of 10 ml/kg
Group II: Acetic acid treated group
Group III: Sulfasalazine (360 mg/kg)[17] + 4% acetic acid
Group IV: EEOC 200 mg/kg +4% acetic acid
Group V: EEOC 300 mg/kg +4% acetic acid
Group VI: EEOC 400 mg/kg +4% acetic acid

Assessment of colonic damage

Animals were euthanized 24 hrs after induction of colitis. 10 cm of the distal colon was removed and assessed for mucosal (macroscopic) injury. They were also processed for histopathological examination.

The grading scale of Morris *et al*. was used for the macroscopic scoring:[18]

Score 0: No damage.
Score 1: Localized hyperemia but no ulcers.
Score 2: Linear ulcer with no significant ulceration.
Score 3: Linear ulceration 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.

The degree of inflammation histopathologically was graded semiquantitatively from 0 to 11 as the sum of:[19]

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

Statistical analysis

Mean ± standard error of mean (SEM) values were calculated for each group. Significant differences between the groups were analyzed using one-way analysis of variance followed by Dunnett's multiple comparison test and results were found to be significant ($p<0.05$) All analysis were done using Graphpad prism software version 5.01.

RESULTS

Results are presented as mean ± SEM of six animals in each group in Figures 1-3 and Tables 1-3.

The weight of the damaged colon tissue is measured and is considered as an indicator of the extent of inflammatory response. The mean colon weight change of Group I was 4.38±0.13, in Group II it was 9.18±0.26, 4.70±0.10 in Group III and in Groups IV, V and VI the values were 7.36±0.11, 6.60±0.05 and 5.45±0.14 respectively. Thus, the maximum increase in weight was in the acetic acid treated group. In the extract treated groups, i.e., Groups IV, V, VI there was dose dependent decrease in the rise of colon weight. However, this decrease in the rise of colon weight in extract treated group was less than the Group III, i.e., the sulfasalazine treated group.

Table 1: Colon weight.

Groups	Treatment	Mean±SEM
I	Normal saline	4.38±0.13*
II	Acetic acid	9.18±0.26
III	Sulfasalazine	4.70±0.10*
IV	EEOC 200 mg/kg	7.36±0.11*
V	EEOC 300 mg/kg	6.60±0.05*
VI	EEOC 400 mg/kg	5.45±0.14*
F	147.9	
df	5,30	

*p<0.01 when compared with the acetic acid treated group (Group II), SEM: Standard error of mean, EEOC: Ethanolic extract of *Oxalis corniculata*

Table 2: Total macroscopic score.

Groups	Treatment	Mean±SEM
I	Normal saline	0.33±0.21*
II	Acetic acid	4.83±0.16
III	Sulfasalazine	1.66±0.21*
IV	EEOC 200 mg/kg	3.66±0.21*
V	EEOC 300 mg/kg	2.83±0.16*
VI	EEOC 400 mg/kg	2.16±0.16*
F	68.54	
df	5,30	

*p<0.01 when compared with the acetic acid treated group (Group II), SEM: Standard error of mean, EEOC: Ethanolic extract of *Oxalis corniculata*

Table 1 and Figure 1 represent the colon weight in the six groups.

Table 2 and Figure 2 represent the macroscopic scores in different groups.

The mean values of macroscopic scoring in Groups I, II, III, IV, V, and VI were 0.33±0.21, 4.83±0.16, 1.66±0.21, 3.66±0.21, 2.83±0.16 and 2.16±0.16 respectively. Thus, the maximum macroscopic damage was in Group II i.e.; acetic acid treated group. In the groups treated with sulfasalazine and extract, the score was a minimum in Group III followed by the highest dose of the extract, i.e., Group VI.

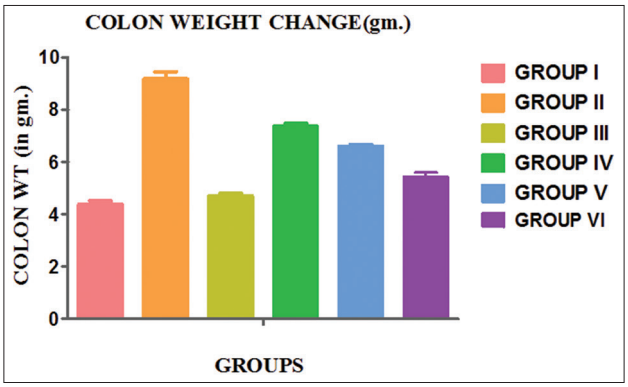

Figure 1: Colon weight change.

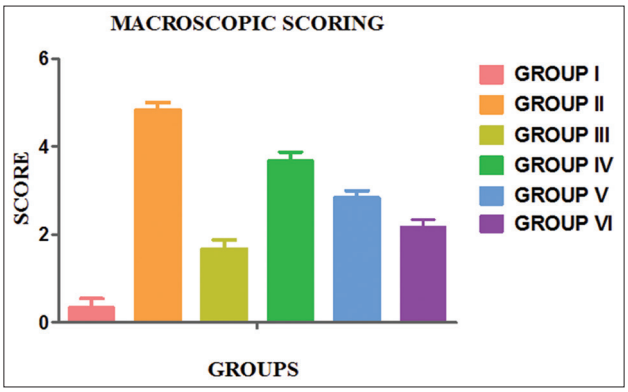

Figure 2: Total macroscopic score.

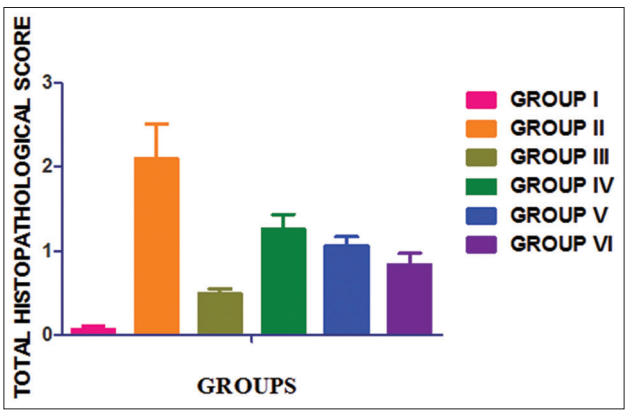

Figure 3: Total histopathological score.

Table 3: Total histopathological score.

Groups	Treatment	Mean±SEM
I	Normal saline	0.0664±0.037*
II	Acetic acid	2.0998±0.409
III	Sulfasalazine	0.4864±0.062*
IV	EEOC 200 mg/kg	1.26±0.175*
V	EEOC 300 mg/kg	1.06±0.109*
VI	EEOC 400 mg/kg	0.84±0.131*
F	10.11	
df	5,30	

*p<0.01 when compared with the acetic acid treated group (Group II), SEM: Standard error of mean, EEOC: Ethanolic extract of *Oxalis corniculata*

Table 3 and Figure 3 represent the total histopathological score which was calculated as the mean ± SEM values of the five parameters as mentioned above. The total histopathological score were 0.0664±0.037, 2.0998±0.409, 2.0998±0.409, 0.4864±0.062, 1.26±0.175, 1.06±0.109 and 0.84±0.131 in Groups I, II, III, IV, V, and VI, respectively. The score was highest in the acetic acid treated group. In the extract treated groups, the score decreased in a dose-dependent manner however it was higher than the Group III, i.e., the group receiving sulfasalazine.

DISCUSSION

The purpose of the study was to evaluate the anti-inflammatory effect of EEOC in IBD induced in rats. Acetic acid was used to induce colitis. Both macroscopic and histologic features were analyzed for assessing the damage to the colon. It was seen that the anti-inflammatory property was maximum with the IBD standard, sulfasalazine. Both the macroscopic and histologic scoring was the least in this group among the various treated groups. Among the extract treated groups the anti-inflammatory property was maximum with the highest dose of the extract i.e., 400 mg/kg. The anti-inflammatory activity of the extract increased in a dose dependent manner. The results of the study show that the colonic damage as determined by histopathology and macroscopically visible damage parallels each other. *O. corniculata* is widely used in Indian traditional medicine as anti-inflammatory, digestive, diuretic, antibacterial and antiseptic. Srikanth *et al.* in their review on *O. corniculata* reveals that wide range of phytochemical constituents have been isolated from the plant such as alkaloids, flavonoids and tannins, phenols and phytosterols. This study also establishes the *in-vitro* anti-inflammatory activity of *O. corniculata*.[20] The study done by Kumari and Sharma also establishes the anti-inflammatory property of *O. corniculata*.[21] The flavonoids and related polyphenols present in the *O. corniculata* Linn. extract may be responsible for the activity.[22] However, there is need for further investigations for identification of the exact mechanism of action responsible for the anticonvulsant activity of *O. corniculata.*

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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Cite this article as: Dutta A, Handique C, Lahkar M. Evaluation of anti-inflammatory activity of *Oxalis corniculata* in experimentally induced inflammatory bowel disease in rats. Int J Basic Clin Pharmacol 2015;4:744-8.