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Pharmacological Study

Protective effect of *Agave americana* Linn. leaf extract in acetic acid-induced ulcerative colitis in rats

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Abstract

Introduction: Natural plants always provide core compounds for new drug development. In the present life and food style, inflammatory bowel disease has become common and needs a lead compound for its drug development. **Aim:** To evaluate the effect of *Agave americana* Linn. leaf extract in acetic acid-induced ulcerative colitis in rats based on its traditional anti-inflammatory use. **Materials and Methods:** Male Wistar rats were pretreated with *A. americana* leaf extract in the dose of 200 and 400 mg/kg p.o. daily for 7 days. On 8th day, 2 ml of 4% v/v acetic acid in saline was instilled into rats' rectum. Prednisolone was used as standard drug and it was administered on the day of acetic acid instillation and continued for 3 days. Extract treatment was continued till 11th day. Body weight, ulcer score, colonic muscle contraction, antioxidant activity and histopathology were studied. Statistical analysis was performed using Parametric one-way analysis of variance followed by Tukey's posttest. **Results:** *A. americana* have retained total body weight significantly ($P < 0.01$) and decreased colon weight/length ratio. Extract have shown a significant decrease ($P < 0.001$) in ulcer scores, myeloperoxidase, lipid peroxidase activity. Further, extract have shown significant improvement in colonic muscle contraction, histopathology of colon etc., which is comparable with standard drug. **Conclusion:** *A. americana* possess protective effect against acetic acid-induced colitis in rats.

Key words: Acetic acid, *Agave americana*, antioxidant, ulcerative colitis

Introduction

Ulcerative colitis is a major form of inflammatory bowel disease (IBD). Ulcerative colitis and Crohn's disease together constitute IBD. Crohn's disease may be localized in any part of the digestive tract and affects the entire intestinal wall whereas ulcerative colitis is confined to the colon and rectum, and the inflammation is restrained to the intestinal lining.[1] IBD is a growing worldwide health burden. Specifically, many developing countries have seen a dramatic rise in the incidence of IBD since 1990.[2] Although etiology of IBD is unknown it appears that an abnormal response of the mucosal innate immune system to luminal bacteria may trigger inflammation that is perpetual by deregulation of cellular immunity.[3-5] Free radicals contribute, aggravate and precipitate pathological changes in the colonic mucosa and have been proposed as therapeutic targets. Localized inflammation, neutrophil infiltration and vicious cascade of generation of inflammatory mediators have been elucidated to damage the colonic mucosal.[6]

IBD, an incurable disease, places a heavy burden on populations because it reduces the quality of life and capacity for work and increases disability. The prevalence of IBD is >200 cases/100,000

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inhabitants in the West and IBD has a major impact on health care resources.[7] The most common classes of medications used in the treatment of IBD include the 5-aminosalicylates (agents), corticosteroids, immunosuppressive agents, antibiotics, and biological agents.[8]

Phyto-botanical and ethno-botanical researches have focused for decades on the search for the single "active principle" in plants, based on the assumption that a plant has one or few ingredients that determine therapeutic effects. But the traditional system of medicine like Ayurveda, traditional Chinese medicine or the European pharmacotherapy generally assumes that a synergy of all ingredients of the plants will bring about the maximum therapeutic efficacy.[9] Reports suggests that 80% drug molecules are natural products or natural compound inspired.[10] The leaves of *Agave americana* Linn. (Family: *Agavaceae*) are used as demulcent, hepatoprotective, antioxidant, antiseptic and to relieve various liver diseases.[11] The sap of the leaves has anti-inflammatory activity and is used internally in case of wounds and inflammation.[12]

The major phyto constituents present in A. *americana* are saponins, that are a vast group of glycosides, widely distributed in higher plants. Pharmacological properties of saponins include anti-inflammatory, antifungal, antibacterial, antiparasitic, and antitumor activity.[13] Hence, the above plant is used to study its effect as anti-inflammatory in acetic acid-induced ulcerative colitis.

To the best of our knowledge, no scientific data regarding the activity of A. *americana* on IBD is available in the literature. Therefore, this work has been undertaken.

Materials and Methods

Drugs and chemicals

Prednisolone, acetic acid, tween 60, sodium carboxymethylcellulose, O-dianisidine hydrochloride, hexadecyl trimethyl ammonium bromide, and trichloro acetic acid was purchased from Hi Media, Mumbai, Maharashtra, India. All chemicals used were of analytical grades.

Plant material and preparation of extract

Fresh leaves of A. *americana* were collected from surrounding areas of Dharwad, Karnataka and authenticated at Department of Botany. The plant material was washed with running water. Fresh leaves of the plant pounded well by using mortar and pestle. The obtained herbal juice was filtered, and the filtrate was subjected to rotary flash evaporator under reduced pressure to dryness. The dried extract was stored in desiccators till use.[14]

Preliminary phytochemical investigation

The A. *americana* leaf extract was subjected to preliminary phytochemical investigation as per standard procedure.[15]

Animals

Male Wistar rats (since female rats show physiological and hormonal changes that may affect results) weighing 150–200 g were used for the present study. For acute toxicity study, female albino mice were used. The animals were procured and maintained in the animal house for experimental purpose. The animals were maintained under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles. They were fed with commercial stock diet and water, *ad libitum*. The animals were housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. Animals were habituated to laboratory conditions for 48 h prior to the experimental protocol to minimize nonspecific stress if any. All the studies conducted were approved by the Institutional Animal Ethics Committee (REG. No. 112/1999/CPCSEA) according to prescribed guidelines of CPCSEA, Government of India.

Experimental method

Determination of acute toxicity

Acute toxicity study was carried out using female albino mice (20–30 g) by up and down/staircase method using OECD 423 guidelines. The A. *americana* leaf extract was orally administered to mice at the doses of 50, 300, 1000, 2000 and 4000 mg/kg body weight respectively. Animals were observed for 48 h to study the general behavior of animals, signs of discomfort and the nervous manifestation. The A. *americana* leaf extract was found devoid of mortality of animals at the dose of 4000 mg/kg body weight. Hence, the 1/10th (400 mg/kg, p.o.) and 1/20th (200 mg/kg, p.o.) of the doses were selected.

Acetic acid-induced colitis in rats

The animals were divided into five groups, each containing 6 rats:

- Group 1: Normal control (treated with vehicle)
- Group 2: Positive control (acetic acid 2 ml of 4% intra-rectally)
- Group 3: A. *americana* extract (200 mg/kg) + acetic acid
- Group 4: A. *americana* extract (400 mg/kg) + acetic acid
- Group 5: Prednisolone (2 mg/kg)[16] + acetic acid.

Agave americana extracts were administered for 7 consecutive days. On the 8th day, overnight fasted animals were anaesthetized using pentobarbitone sodium and 2 ml of acetic acid (4% v/v in 0.9% saline) was instilled into the rectum. Animals were allowed to hang in air by holding their tails for 1–2 min. This prevents spillage of solution from rectum. Extract and standard drug treatment were continued till 11th day. On 11th day, animals were weighed and sacrificed and dissected to remove colon. Inflammation was assessed based on physical parameters, macroscopy and microscopic features. Quantification of inflammation was done using biochemical assay.[16,17]

Physical parameters assessed

Change in:

- Body weight
- Colon weight
- Colon length
- Colon weight/length ratio.

As the weight loss is one of the clinical symptoms, all the rats from respective groups were weighed each day, and the percentage of the original weight is used for evaluation.[18-20]

Ulcer scoring for colon

For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, and slightly cleaned in physiological saline to remove fecal residues. Pieces of colon (10 cm long each) were scored for macroscopic features using scoring pattern.[16]

Antioxidant activity

Myeloperoxidase and lipid peroxidase assay

The colitis caused by acetic acid was associated with an

increase in myeloperoxidase (MPO) and lipid peroxidase (LPO) activity.[16,18]

Colonic muscle contraction activity

Tissue preparation

After a laparotomy incision, a portion of the colon was removed and placed in an oxygenated Tyrode's solution. A segment of 2 cm length colon were mounted in a 10 ml organ bath containing Tyrode's solution that was bubbled with a 95% O_2 and 5% CO_2 mixture and the temperature was held at 37°C.

Measurement of smooth muscle contractility

Each segment was allowed to equilibrate in the bath for 30 min. Muscle contraction was measured using 10 μg solution of acetylcholine in student's organ bath apparatus. Different responses were recorded on kymograph and percentage response was calculated for each group.[21]

Evaluation based on microscopic (histological) characters

The colon from each animal was removed after sacrificing the animal and collected and preserved in 10% formalin solution. The samples were studied for histological changes.

Statistical analysis

All data was expressed as mean ± standard error of the mean of 6 rats per experimental group. Statistical analysis was performed using the GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, USA) statistical software. Parametric one-way analysis of variance is followed by Tukey's posttest. The minimal level of significance was identified at $P < 0.05$.

Results

The phytochemical investigation of *A. americana* leaf extract showed the presence of steroids, triterpenoids, saponin glycosides, flavonoids, alkaloids and carbohydrates.

Evaluation based on body weight shows that there was significant ($P < 0.001$) decrease in the body weight of induced group compared with the normal group [Figure 1]. The animals treated with 200 mg/kg, 400 mg/kg leaf extract and the standard group could retain their total body weight compared with the positive control group [Table 1]. The acetic acid induction significantly ($P < 0.001$) increased the colon weight of positive control group compared to the normal group. Whereas the animals treated with 200 mg/kg, 400 mg/kg extract ($P < 0.01$) and standard group ($P < 0.001$) decreased the colon weight significantly compared with positive control group [Table 2]. The disease induction by instillation of acetic acid intra-rectally causes significant ($P < 0.001$) shrinkage in the colon length of the positive control group compared to normal control. The animals treated with 200 mg/kg, 400 mg/kg extract ($P < 0.01$) and standard group increased the colon length significantly ($P < 0.001$) compared with positive control group [Table 2].

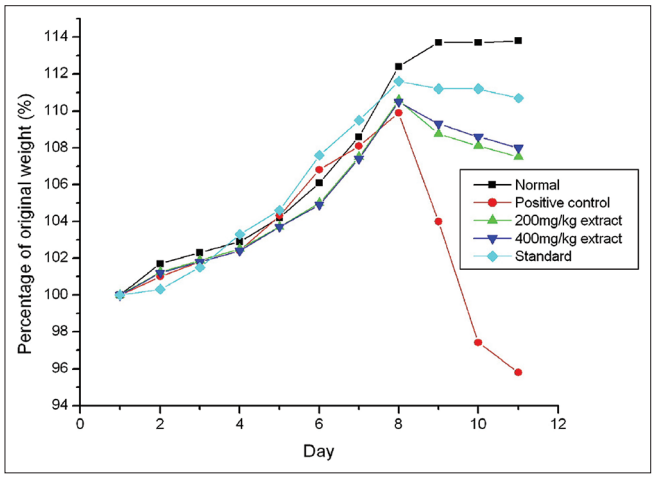

Figure 1: Effect of *Agave americana* leaf extract on body weight in acetic acid-induced colitis

The colon weight/length (mg/cm) ratios was significantly ($P < 0.001$) increased in positive control group compared to normal control group. Whereas the animals treated with 200 mg/kg, 400 mg/kg extract and standard group significantly reduced ($P < 0.001$) the colon weight/length ratio [Table 2].

Intra-rectal instillation of acetic acid caused an inflammatory reaction in the colon. The inflammation covered rectum and distal colon portion. The visible changes include severe epithelial necrosis and ulcerated mucosa. Evaluation based on macroscopic features showed score values significantly increased ($P < 0.001$) in the positive control as compared with normal control group. The treatment with plant extract 200 mg/kg, 400 mg/kg and standard drug showed significantly ($P < 0.001$) decreased score values as compared to the positive control group. The values obtained for the drug-treated group (especially 400 mg/kg) was comparable with that of standard treated group [Table 3].

The colitis caused by acetic acid was associated with an increase in MPO activity. The MPO assay showed significant ($P < 0.001$) increase in MPO activity of positive control (group compared to normal control group). The animals treated with extract and standard group showed significant ($P < 0.001$) decrease in MPO activity compared to the positive control group [Table 3]. The LPO assay showed significant ($P < 0.001$) increase in LPO activity of positive control group compared to normal control group. The animals treated with plant extract, and standard group showed significant ($P < 0.001$) decrease in LPO activity compared to the positive control group [Table 3]. The colonic muscle contractions were recorded using acetylcholine and Tyrode solution showed a significant decrease in percentage response compared to normal control group, which indicates severe damage to colonic tissue and receptors [Table 4]. On the other hand, there was a significant increase in percentage response in standard and drug-treated groups compared to the positive control [Figure 2].

Histological examination of positive control group showed massive necrosis of the mucosa and sub mucosa. Test groups (200 and 400 mg/kg body weight) showed mild lesions, regeneration and inflammatory reaction. The standard group showed suppressed inflammatory reaction [Figure 3a-e].

Discussion

Ulcerative colitis is a refractory, chronic, and nonspecific disease occurring usually in the rectum and the entire colon. The primary aims of medical therapy for patients with

Table 1: Effect of *Agave americana* leaf extract on body weight in acetic acid-induced colitis

Group	Body weight (g)		Percentage change in body weight (%)
	Initial (1st day)	Final (11th day)	
Normal	158.33±2.813	180.3±2.716	8.96 ↑
Positive control	159.2±3.124	152.5±3.354***	11.55 ↓
200 mg/kg extract	160±2.887	172±2.757##	7.31 ↑
400 mg/kg extract	161±3.950	174±4.163###	7.57 ↑
Standard	162.5±3.594	180±3.715###	9.16 ↑

Each value is expressed as mean±SEM for six animals in each group. ***Significant decrease in body weight $P<0.001$ with respect to normal group, ###Significant increase in body weight $P<0.001$ with respect to positive control group, ##Significant increase in body weight $P<0.01$ with respect to positive control group. SEM: Standard error of mean

Table 2: Effect of *Agave americana* leaf extract on colon weight (g), length (cm) and weight/length ratio (mg/cm) in acetic acid-induced colitis

Groups	Colon weight (g)	Colon length (cm)	Colon weight/length ratio (mg/cm)
Normal	1.248±0.02	11.82±0.2	105.8±3.13
Positive control	1.723±0.05***	9.88±0.17***	174.2±4.659***
200 mg/kg extract	1.418±0.049##	11.25±0.23##	126.2±4.58###
400 mg/kg extract	1.363±0.02##	11.38±0.25##	120±3.06###
Standard	1.327±0.09###	11.63±0.39###	114.1±7.53###

***Significant increase in colon weight $P<0.001$ compared to normal group, ###Significant decrease in colon weight $P<0.001$ compared to positive control group, ##Significant decrease in colon weight $P<0.01$ compared to positive control group

Table 3: Effect of *Agave americana* leaf extract on ulcer score, MPO activity and LPO assay in acetic acid-induced colitis

Groups	Ulcer score	MPO activity (U/g)	LPO assay (U/g)
Normal	0	0.9602±0.09	0.3798±0.02
Positive control	4.33±0.5***	4.18±0.18***	0.77±0.03***
200 mg/kg extract	1.83±0.4##	2.19±0.05###	0.547±0.02###
400 mg/kg extract	1.5±0.34###	1.52±0.05###	0.512±0.02###
Standard	0.83±0.47###	1.29±0.07###	0.4675±0.02###

***Significant increase in score values $P<0.001$ compared to normal group, ###Significant decrease in score values $P<0.001$ compared to positive control group, ##Significant decrease in score values $P<0.01$ compared to positive control group. MPO: Myeloperoxidase, LPO: Lipid peroxidase

Table 4: Effect of *Agave americana* leaf extract on colonic muscle contractility using acetylcholine (10 µg) and Tyrode's solution in acetic acid-induced colitis

Dose of ACh in ml	Percentage response				
	Normal	Positive control	200 mg/kg extract	400 mg/kg extract	Standard
0.1 ml	22.22	11.11	18.5	18.5	18.5
0.2 ml	48	22.22	29.6	29.6	37
0.4 ml	74	33.3	44.4	44.4	51.85
0.8 ml	100	33.3***	55.5	70.3	77.7
1.6 ml	100###	-	55.5##	70.3###	77.7###

***Significant decrease in percentage response compared to normal group, ###Significant increase ($P<0.001$) in percentage compared to positive control group. ##Significant increase ($P<0.01$) in percentage compared to positive control group. Ach: Acetylcholine

ulcerative colitis are directed at inducing and then maintaining remission of symptoms and mucosal inflammation to provide an improved quality of life with the least amount of steroid exposure.[22] According to traditional claims, *A. americana* leaf exhibits anti-inflammatory, wound healing and antioxidant property.[11,23]

Decrease in body weight is one of the symptoms of ulcerative colitis. The present studies have shown a significant decrease in body weight of positive control group compared to normal control group. There was a significant increase in body weight in extract treated groups showing its protective effect in ulcerative colitis.

Other findings with IBD are abnormal histopathological features and increased intestinal permeability. Results have also shown the shrinkage of colon and increase in colon weight in positive control group, which indicates severe inflammation along with increased intestinal permeability. *A. americana* extract treated animals have shown a significant decrease in shrinkage and weight of the colon indicating its anti-inflammatory effect.

Mediators like tumor necrosis factor-alpha, interleukin-6 (IL_6), and LB_4 plays an important role in the inflammation of the intestinal mucosa in animal models of IBD. Results have shown a significant decrease in ulcer score of extract treated animals compared to positive control group proving its beneficial effect in ulcerative colitis. In IBD, the inflammatory process is probably derived from the chronic presence of numerous, activated MPO-containing phagocytes in the inflamed intestine.

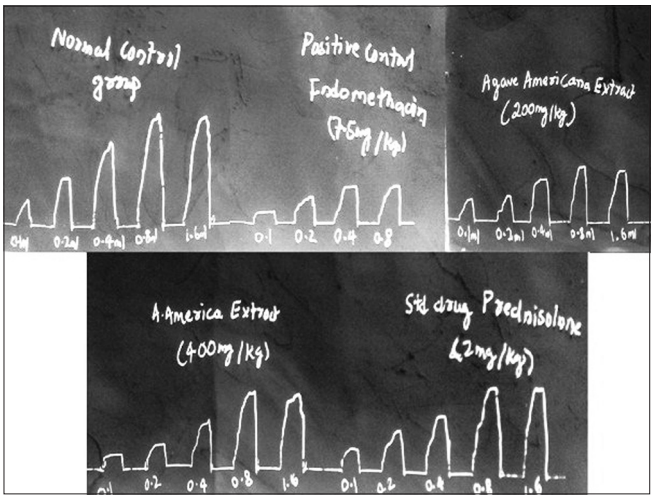

Figure 2: Effect of *Agave americana* leaf extract on colonic muscle contractility using acetylcholine (10 µg) and Tyrode solution in acetic acid-induced colitis

MPO assay has provided data supporting protective effect of the extract showing significant decrease in MPO activity in the extract treated groups compared to the positive control.

Oxidative stress also plays an important role in the pathophysiology of IBD.[24] In patients with IBD, especially ulcerative colitis, the repeated cycle of injury of the intestinal mucosa has been shown to increase the risk of colon

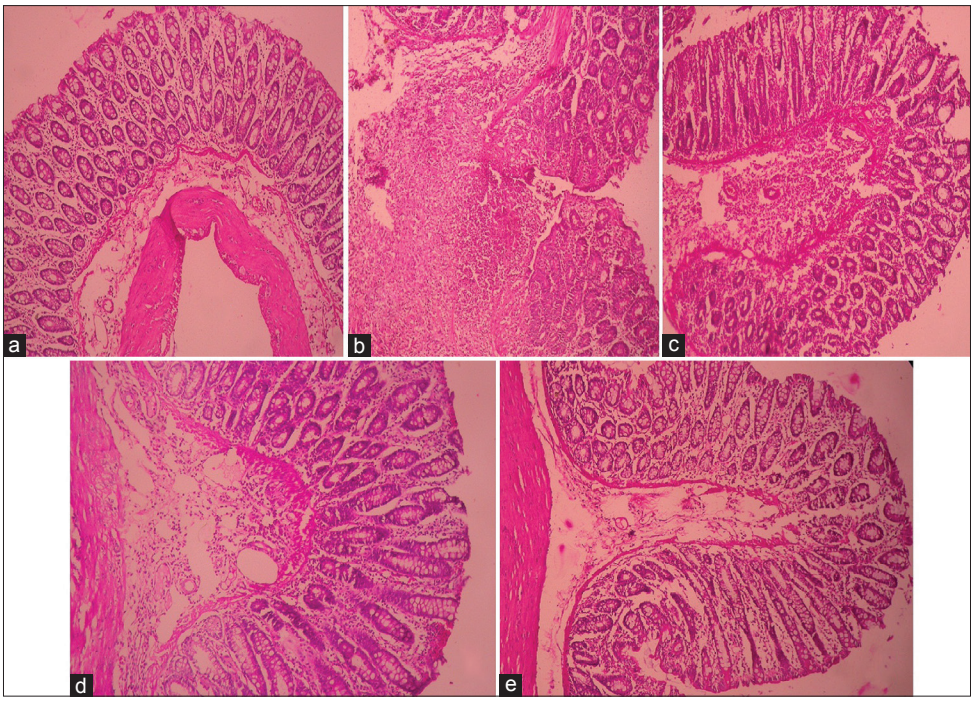

Figure 3: Histopathological examination of colon (colonic mucosal sections): (a) Normal rat showing normal mucosa with intact epithelial surface, (b) Acetic acid-induced colitis showing massive necrotic destruction of epithelium, (c-d) Treatment with extract of *Agave americana* leaf 200 mg/kg and 400 mg/kg showing decreased epithelial damage, regeneration and suppressed inflammatory reaction, (e) Standard group showed suppressed inflammatory reaction

cancer.[25] LPO assay is an indicator of oxidative stress and reactive metabolites. Results have shown there was a significant increase in LPO activity in acetic acid-induced group whereas significant decrease in LPO activity has been observed in the extract and standard drug-treated groups.

Dose response curve of acetylcholine (Ach) using isolated rat colon from each different group has been carried, which has provided data supporting beneficial effect of A. *americana* in ulcerative colitis. There was a significant decrease in % response in an induced group indicating severe necrosis, damage and ulcer in the colon that leads to decrease in a number of muscarinic receptors hence reduced % response by Ach. On the other hand, extract and standard drug-treated groups have shown a significant increase in % response indicating healing and protective effect in acetic acid-induced ulcerative colitis.

Histopathological studies have shown severe necrosis and edema in induced group. Whereas extract treated groups have shown intact intestinal mucosa along with regeneration of intestinal epithelium cells providing protective mechanism.

In all above studied parameters, extract treated groups have shown better results that are comparable with the standard prednisolone treated group. And the possible mechanism of action may be by decreasing the number of neutrophils and reduction in the synthesis of inflammatory mediators and by increasing protective and regenerative property.

Conclusion

Agave americana leaf extract has significant protective effect against acetic acid-induced colitis models. This investigation has opened avenues for the treatment of IBD from the A. *americana*.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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हिन्दी सारांश

चूहों में एसिटिक ॲसिड प्रेरित अलसरेटिव कोलाइटिस में अगेव अमेरिकाना पत्र सत्व का सुरक्षात्मक प्रभाव

बशीरअहमद ए.ए. मननसाहेब, प्रीति व्ही. कुलकर्णी, मशूद अहमद सांग्रेसकोप्प, चेतन सावंत, अंजना मोहन

प्रस्तुत अध्ययन, चूहों में एसिटिक ॲसिड प्रेरित अलसरेटिव कोलाइटिस में अगेव अमेरिकाना पत्र सत्व का सुरक्षात्मक प्रभाव जानने हेतु किया गया। पुरुष विस्टार चूहों को ७ दिनों तक २०० और ४०० मि.ग्रा./कि.ग्रा. प्रति दिन के अनुसार एगेव अमेरिकाना के पत्तों का सत्व मुख द्वारा दिया गया। ८ वें दिन सलाइन मिश्रित २ मि.ली. (४% व्ही/व्ही) एसिटिक ॲसिड चूहों की मलाशय में टपकाया गया। प्रेडनिसोलन सर्वस्वीकृत मानक दवा के रूप में इस्तेमाल कि गयी और यह एसिटिक ॲसिड टपकने के दिन प्रशासित की गयी और ३ दिन के लिए जारी रखी गयी। पत्तों का सत्व ११ दिन तक दिया गया। शरीर के वजन, अलसर स्कोर, बृहदांत्र संबंधी मांसपेशियों में संकुचन, एंटीऑक्सीडेंट गतिविधि और ऊतकविकृतिविज्ञानी अध्ययन किया गया। ए अमेरिकाना पत्तों के सत्व ने चूहों के शरीर का कुल वजन (पी<0.01) बनाए रखा और बृहदान्त्र के वजन/लंबाई के अनुपात में कमी पायी गयी। पत्तों के सत्व इलाज से अलसर स्कोर, एंटीऑक्सीडेंट गतिविधि (पी<0.001) मे काफी कमी पायी गयी। इसके अलावा, बृहदांत्र संबंधी मांसपेशियों में संकुचन और ऊतकविकृतिविज्ञानी अध्ययन में महत्वपूर्ण सुधार देखा गया है; जो कि प्रेडनिसोलन दवा के साथ तुलनीय है। इस से अगेव अमेरिकाना चूहों में एसिटिक ॲसिड प्रेरित अलसरेटिव कोलाइटिस के खिलाफ सुरक्षात्मक प्रभाव सिद्ध होता है।