Section A-Research paper

Curative Effect of *Caesalpinia Pulcherrima* Leaf Extract on Acetic Acid Induced Ulcerative Colitis in Sprague Dawley Rats

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Abstract

Background and Objectives: Ulcerative colitis is an inflammatory bowel disorder caused by a dysregulated immune response to the gut microbiota, which is influenced by a combination of environmental and genetic variables. TNF-α stimulates the inflammatory response by inducing a cascade of immune responses, including an increase in pro-inflammatory mediators and proteases, activation of chemotaxis, and infiltration of inflammatory cells, all of which lead to ulceration and bleeding via cytotoxic reactive oxygen species. *Caesalpinia pulcherrima* has been shown to have anti-inflammatory and antioxidant properties. In this study, the curative effect of CPE on ulcerative colitis in rats is assessed.

Materials and Methods: Rats were randomly divided into 5 groups (n = 6) and received daily oral administration of normal saline (10 mL / kg), sulfasalazine (500 mg / kg), or CPE (200 and 400 mg / kg) on day 0. Until the 7th day. Colitis was produced on day 4 by administering 500 μl acetic acid (4 percent v/v) intrarectally. Graph pad prism (ver.09) was used for statistical analysis.

Results: In the untreated colitis group, serum glutathione (GSH) levels decreased, while colonic malondialdehyde (MDA), colonic myeloperoxidase (MPO), serum interleukin 6 (IL-6), and serum tumor necrosis factor(TNF-) increased significantly. All of these indicators were significantly improved by CPE treatment, particularly at the 400 mg/kg dose ($p < 0.001$), with no significant change between the 500 mg/kg sulfasalazine group and the normal group.

Conclusion: CPE demonstrated protection against acetic acid-induced ulcerative colitis, possibly due to its anti-inflammatory and antioxidant actions.

Keywords: Anti-ulcerative colitis, anti-inflammatory, *Caesalpinia pulcherrima*, MDA, Gut, IBD, Antioxidant.

Introduction

Ulcerative Colitis(UC) is a chronic inflammatory bowel disorder (IBD) characterized by persistent mucositis, ulceration, abdominal pain, haemorrhage, and bloody stools[1]. The aetiology of IBD is still unknown, but there are several environmental, genetic, immune and reactive oxygen species involved in the pathogenesis of IBD[2,3]. Different drugs are currently used for the treatment of UC, including aminosalicylates, immunosuppressants, corticosteroids, and biological therapies such as TNF-α antibodies. Although they have all shown some degree of efficacy in UC, their long-term use is limited due to the frequency and severity of side effects, cumbersome dosing regimen, and partially prohibitive price[4,5]. As a result, a key priority in human UC therapy is the development of new medications that combine efficacy, ease of dosage, and fewer adverse effects. In this sense, alternative therapies have become a popular treatment option for gastrointestinal ailments; in fact, research shows that nearly half of IBD patients have used or are currently using complementary therapies[6,7]. There are many different types of alternatives and or Complementary therapies, although botanical medications are very relevant for the treatment of intestinal inflammation. This may be mainly related to their safety, since they have taken from antiquity, in addition to its reputation efficacy, probably due to the presence of different active ingredients components that can simultaneously target multiple pathways or mediators of the inflammatory response[8]. Experimental colitis models have been used to identify therapeutic agents and elucidate the underlying physiological mechanisms of UC. Acetic acid colitis widely used model recapitulates the histological characteristics of UC[9,10].

Caesalpinia pulcherrima, belonging to the family Caesalpiniaceae, is an ornamental plant due to its diversity of flowers, which appear yellow, pink, whitish, and red with yellow edges. Patag is the common name for it. In English known as C. pulcherrima wood, wood from Brazil. *C. pulcherrima* is distributed in Andhra Pradesh, Karnataka, Tamilnadu, Kerala and West Bengal[11]. Phytochemical studies reveal the presence of various phytoactive ingredients such as pulcherrimine, terpenoids, sitosterol, retinoids, flavonoids such as quercetin and myricetin, carotenoids, glycosides, steroids, and phenols. Traditionally, the plant has been used to treat gastritis, bronchitis, inflammation, malaria infection, and abortifacient. In addition, anti-arthritic, anti-inflammatory, anticancer, antidiabetic, immunosuppressive and antimicrobial activities have also been reported as properties of this plant[12]. However, no research has been done on *C. pulcherrima*. As a result, the goal of this study was so that *C. pulcherrima* could protect rats from ulcerative colitis caused by acetic acid.

Materials and Methods:

Study area:

The study was carried out in the Pharmacology Department, PRRM Collegeof Pharmacy, Kadapa, India from June 2019 to January 2020.

Plant collection and Extract preparation:

Fresh *Caesalpinia pulcherrima* leaves were collected from the Tirupathi hill forest in Chittoor district of Andhra Pradesh, India. The botanical identity was confirmed at the SV University, Tirupathi, Department of Botany by a plant taxonomist. For seven days, the plant material was air-dried. Three and a half kilograms (3.5 kg) of the dried material was pulverized into a fine powder using a high-power mixer. The fine powder was soaked with 5 L of 70% (v/v) ethanol for 72 hours, filtered and concentrated on a rotary evaporator at 50°C, and solidified in an oven. The wet gummy solid extract was kept in a desiccator for 72 h. For oral administration, the extract was reconstituted as an emulsion in 2% tragacanth and designated *Caesalpinia pulcherrima* extract (CPE) in this study.

Animals and Drugs:

Raghavendra enterprises provided 30 male Sprague-Dawley rats 10 to 12 weeks of age and weighing 250 to 300 g.(Bangalore, India). During the whole testing session, they were handled and housed in standard laboratory circumstances in polypropylene cages with no more than six rats per cage at room temperature (25±2 °C) with a 12-hour light-dark cycle and given standard food with water ad libitum. Sulfasalazine, acetic acid (Sigma), and all other factors are analytical grade. Diagnostic kits used in this investigation were provided by Span Diagnostics Ltd., India, and Excel Diagnostics Ltd., India. The current study was authorised by the P. Rami Reddy Memorial College of Pharmacy's Institutional Animal Ethical Committee (1423/PO/Re/S/11/CPCSEA).

Induction of Colitis:

Rats were randomly divided into 5 groups (n = 6) and received daily oral administration of normal saline (10 mL / kg), sulfasalazine (500 mg / kg), or CPE (200 and 400 mg / kg) on day 0. Until the 7th day. Colitis was produced on day 4 by administering 500 μl acetic acid (4 percent v/v) intrarectally. On the eighth day, rats were killed by cervical dislocation under anesthesia with 50 mg/kg pentobarbital (i.p.).

Colon mucosal damage index (CMDI):

The colon tissue of the sacrificial rats was extracted, opened lengthwise, cleaned in a saline buffer, and pinned to a wax block 10 cm near the anus. According to Bell et al.[13] CMDI in each colon was evaluated separately by two independent observers who were unaware of the treatment .

Disease Activity Index (DAI):

Colonic inflammation was evaluated by disease activity index (DAI) after acetic acid infusion. The DAI score was determined based on the methods of Friedman et al.[14]

Biochemical determination of colon inflammation

Colonic myeloperoxidase (MPO):

Lefkovitz et al.[15] proposed a method for measuring colonic myeloperoxidase (MPO). 100 mg of colonic mucosa scrapings were homogenised in 0.5 percent HTA bromide, diluted in KPO_4 buffer (50 mM, pH 6) and sonicated in an ice bath for 10 seconds. Three times the uniform mixture was frozen and centrifuged (20,000 rpm, 15 min). After that, 0.1 ml of supernatant was mixed with 2.9 ml of KPO_4 buffer (50 mM) containing extra H_2O_2 and odionicidine dihydrochloride. A spectrophotometer (Beckman DU 640B) was used to measure the absorbance of this solution at 460 nm for 5 minutes.

Colonic malondialdehyde (MDA):

Ohkawa et al.[16] established the method of measuring colonic malondialdehyde (MDA). After freezing for 15 min, 2 ml of the colonic mucosa was centrifuged at 20,000 rpm with homogeneous TCA (10% w / v). Then 2ml TCA was again mixed with 2ml supernatant. Heat the solution to 100° C for 10 min and then cool rapidly to 0 C for 5 min. At a wavelength of 535 nm, concentration is measured.

Determination of inflammatory markers and antioxidants in serum:

Serum was separated by centrifugation (4°C) at 3000 rpm after collection of blood into clot activator tubes. IL-6 and TNF levels were determined using widely available ELISA kits. Colourimetric kits were used to measure serum CAT, LPO and GSH according to the manufacturer's protocol.

Histopathological Studies:

Colon tissue from the excised colon was promptly preserved in a 10% neutral buffered formalin solution for histological investigations. Serial sections of the paraffin-embedded fixed tissues were taken. Hematoxylin and eosin were used to stain each segment (H&E stain). Under an optical microscope, the sections were examined and photomicrographs were taken.

Statistical Analysis:

All the data wereexpressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Tukey test using computer-based fitting program (Prism graph pad 9). Statistical significance was set accordingly.

Results:

Effect on CMDI and DAI:

Table 1 shows the effect of CPE on colon mucus damage score and disease activity index. Compared to the normal group, the control group had a much higher score for colonic mucus lesions. Compared to the control group, rats receiving CPE at both doses (200 mg/kg and 400 mg/kg) showed a significant reduction in colonic mucus damage score. The DAI score was estimated by adding the scores for weight loss, diarrhoea, and hematochezia. Compared to the control group, animals in test group 1 receiving CPE (200 mg/kg) showed a significant decrease in DAI. Animals in test group 2, given CPE at a dose of 400 mg/kg, showed a substantial reduction in DAI when compared to the control group.

Table 1.Effect of CPE on CMDI and DAIin acetic acid-induced colitis rats

Treatment (Group)	**CMDI Score**	**DAI**
Normal	0.0	0.500 ± 0.28
Acetic acid: Untreated	7.500 ±0.28 $^{###}$	7.963 ±0.13$^{###}$
Sulfasalazine	0.750 ±0.25 ***	3.953 ±0.13***
200 mg/kg CPE	6.250 ± 0.25 *	6.018 ± 0.08**
400 mg/kg CPE	3.250 ± 0.25 ***	4.150 ± 0.08***

[1]All values are shown as mean ± SEM. ### indicates$p$<0.001 when compared to the normal group. **indicate p<0.01, when compared to the control group. *** indicate p<0.001 when compared to the control group.

Effect on Biochemical Parameters:

In the acetic acid-induced colitis group, colonic MPO and colonic MDA levels increased significantly compared with the normal group, as shown in Table 2. Compared with the untreated colitis group, all treatment groups, except for a low dose of CPE (200 mg/kg), significant changes in the restoration of these parameters. This means that, compared to a high dose of CPE, the low dose was less effective. In the acetic acid colitis group, serum levels of TNF-α and IL-6 were significantly increased compared with the normal group. Furthermore, compared to the untreated acetic acid-induced colitis group, all groups had significantly lower serum levels of TNF-α and IL-6. Intergroup pairwise comparisons showed no significant difference in serum TNF-α between normal, sulfasalazine (500 mg/kg), and CPE (400 mg/kg) groups, indicating a near normalization of serum TNF-α by sulfasalazine and high dose CPE (400 mg/kg).

Table 2.Effect of CPE on Biochemical parameters in acetic acid-induced colitis rats

Treatment (Group)	**Colonic MPO (U/g)**	**ColonicMDA (mmol/mg)**	**Serum TNF-α (pg/ml)**	**Serum IL-6 (pg/ml)**
Normal	0.211 ± 0.005	17.23 ± 0.62	18.76 ± 0.63	12.71 ± 0.66
Aceticacid: Untreated	0.477 ± 0.016$^{###}$	48.05 ± 1.81$^{###}$	51.52 ± 3.33$^{###}$	45.99 ± 2.42
Sulfasalazine	0.325 ± 0.017***	15.41 ± 0.99***	20.35 ± 0.91***	12.58 ± 0.41
200 mg/kg CPE	0.407 ± 0.013*	30.9 ± 0.98**	39.98 ± 0.64*	34.55 ± 0.96
400 mg/kg CPE	0.339 ± 0.017***	18.10 ± 0.63***	18.71 ± 1.74***	14.93 ± 0.65

[2]All values are shown as mean ± SEM. ### indicates$p<0.001$ when compared to the normal group. **indicate $p<0.01$, when compared to the control group. *** indicate $p<0.001$ when compared to the control group.

Effect on Anti-Oxidant Parameters

As depicted in Table 3, Acetic acid administration reduced blood antioxidant status by decreasing catalase, GSH levels and increasing LPO levels in the untreated groups compared to the normal group. Both doses of ethanolic extract of *C.pulcherrima* showed a significant increase in the antioxidant status in the blood by increasing catalase levels, GSH and decreasing LPO levels, which were shown to be dose-dependent compared to the control group.

Table 3.Effect of CPE on anti-oxidant parameters in acetic acid-induced colitis rats

Treatment (Group)	**CAT (H_2O_2consumed/gram tissue)**	**GSH (µg of GSH/mg)**	**LPO (µM /mg)**
Normal	27.38 ± 0.59	9.026 ± 0.45	11.64 ± 0.74
Aceticacid: Untreated	15.99 ± 0.66$^{###}$	4.507 ± 0.29$^{###}$	26.09 ± 0.40 $^{###}$
Sulfasalazine	25.42 ± 1.75***	8.204 ± 0.22***	14.63 ± 0.76***
200 mg/kg CPE	22.76 ± 1.09**	6.361 ± 0.29**	20.46 ± 1.32 **
400 mg/kg CPE	24.95 ± 1.10***	7.382 ± 0.25***	13.97 ± 0.70***

[3]All values are shown as mean ± SEM. ### indicates$p<0.001$ when compared to the normal group. **indicate $p<0.01$, when compared to the control group. *** indicate $p<0.001$ when compared to the control group.

Histopathological studies:

Histopathological studies of the normal group of animals showed normal cytoarchitecture of the colon. AA-induced group of animals showed degenerative changes like distractive mild congestion and structural damage in the colon. In a standard group of animals showed regenerative changes in the colon which shows similar to normal tissue. Treatment with CPE (200mg/kg p.o) shows regenerative changes in cytoarchitecture of colon tissue. Treatment with CPE (400mg/kg p.o) shows regeneration of tissue similar to normal cytoarchitecture of the colon.

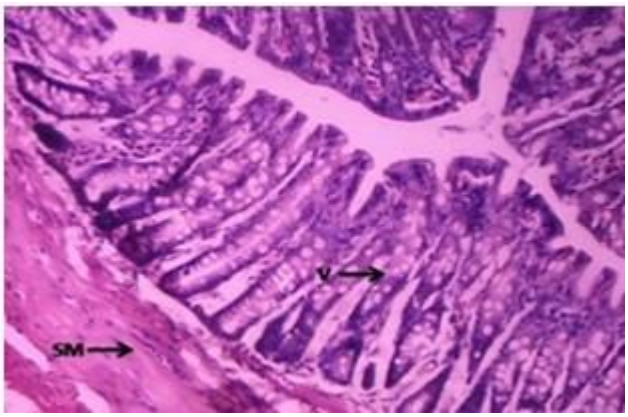
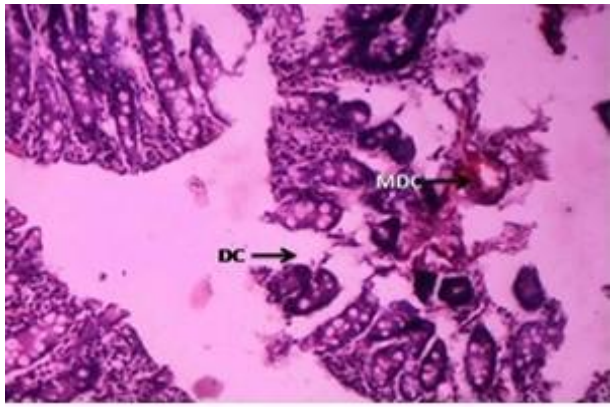
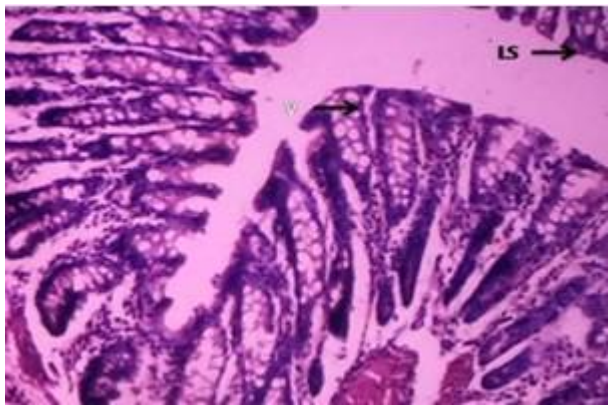


Fig. 1a: Normal rat colon histology shows normal cytoarchitecture of the colon represents as vehicle-treated, normal group.
Stain: Hematoxylin/eosin, Magnification: ×100

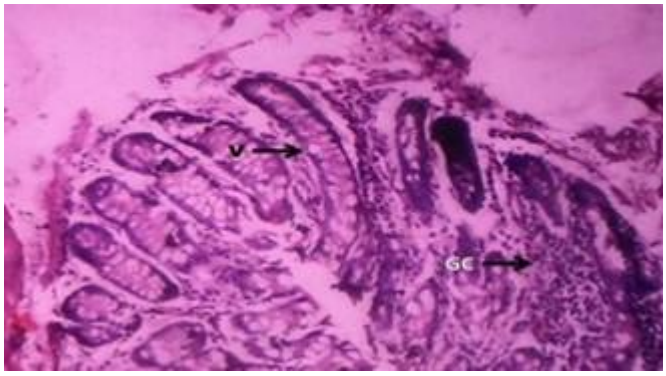


Fig, 1b: AA-induced group of animals shows degenerative changes like distractive mild congestion and structural damage in the colon represents as acetic acid-treated, untreated group.
Stain: Hematoxylin/eosin, Magnification: ×100



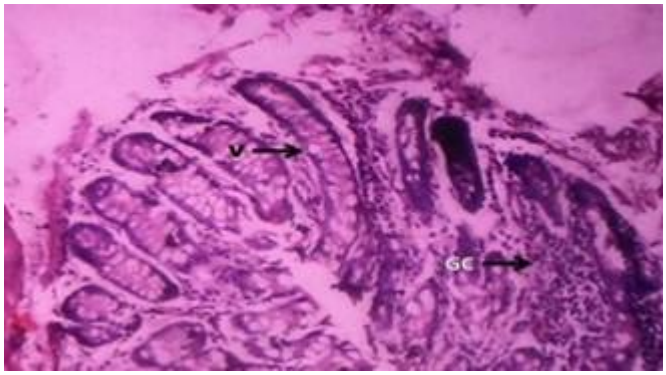
Fig, 1c: standard group of animals shows regenerative changes in the colon which shows similar to normal tissue represents as sulfasalazine treated, standard group.

Stain: Hematoxylin/eosin, Magnification: ×100



Fig, 1d: Colon of rats shows regenerative changes in cytoarchitecture of colon tissue represents as 200 mg/kg ECP-treated groups.

Stain: Hematoxylin/eosin, Magnification: ×100

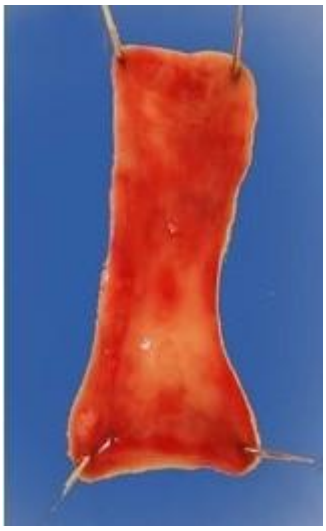


Fig, 1e: Colon of rats shows regenerative changes in cytoarchitecture of colon tissue represents as 400 mg/kg ECP-treated groups.

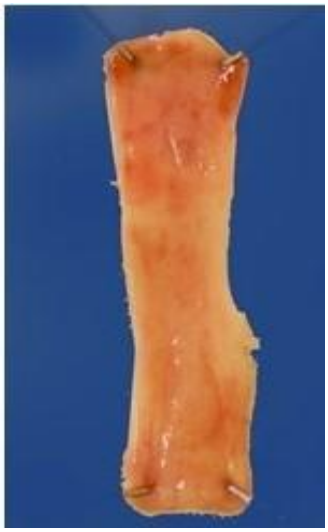
Stain: Hematoxylin/eosin, Magnification: ×100

Colon Images:

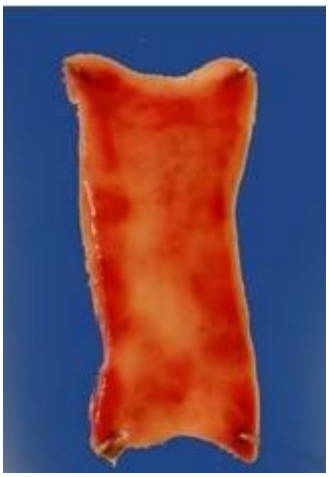
Fig. 2a: Normal rat colon Images shows normal colour of the colon represents as vehicle-treated, normal group.



Fig, 2b: AA-induced group of animals showsdeep reddish colour of colon indicates the presence of inflammation represents untreated group.



Fig, 2c: Standard group of animals shows normal colour in the colon which shows similar to normal tissue represents as standard group.



Fig, 1d: Colon of images of rats shows moderate reddish colour of colon tissue represents as 200 mg/kg ECP-treated groups.

Fig, 1e: Colon of images rats shows changes to normal colour colon tissue which shows similar to normal tissue represents as 400 mg/kg ECP-treated groups.

Discussion:

Ulcerative colitis is a type of inflammatory bowel disease (IBD) that causes long-term inflammation of the intestine. The inner most lining of the large intestine (colon) and the rectum are commonly affected by ulcerative colitis. This only occurs when continuous tracts of the colon appear any where in the digestive tract and then extend deep into the affected tissues[17]. Acetic acid-induced colitis is a well-studied approach to establishing an animal model of UC because it resembles the pathogenesis of UC in humans, with an increase in inflammatory mediators, localized involvement and lesions of theintestinal epithelium[18]. Activation against the ulcerative colitis of *Caesalpinia pulcherrima* leaf extract was assessed by measuring the following parameters such as CMDI, DAI, myeloperoxidase levels, malondialdehyde, inflammatory parameters, histopathological studies and tissue antioxidant

levels. The results obtained in the present study confirm the therapeutic efficacy of CPE when administered at corresponding doses in a sample of colitis induced by acetic acid.

After intracolonic administration of 4% (v/v) acetic acid, the colon showed an inflammatory response characterized by widespread destruction of the mucosal epithelium due to significant free radical activity in colonic tissues, such as suggested reference[19]. Acetic acid-induced induced mucosal and submucosal inflammation of UC followed the initial injury and was associated with the activation of arachidonic acid pathways. The CPE study showed a dose-dependent response to treatment with *Caesalpinia pulcherrima* leaf extract for macroscopic and microscopic scores. Phytochemical study of *Caesalpinia pulcherrima* leaf extract has previously revealed the presence of pulcherrimine, terpenoids, sitosterol, retinoids, flavonoids such as quercetin and myricetin, carotenoids, glycosides, steroids, and phenols[20], which may be responsible for healingcolon ulcers. Animal faecal occult blood from acetic acid-induced induced UC showed additional evidence of ulceration and bleeding in control and disease treatment groups. Occult blood in the faeces of the animals was visible in the disease control group, but not visible in the CPE 400 mg/kg and sulfasalazine dose group. There was a dose-dependent effect with a response similar to the sulfasalazine treated group [21].

In our investigation, the untreated colitis group showed a substantial increase in colonic MPO activity, indicating neutrophil infiltration and dysfunction of the inflammatory cascade, which was observed not only in animal models but also in patients with IBD. MDA and antioxidant levels in the colon also increased, indicating increased damage from oxidative stress, which is consistent with previous research. In our investigation, rats with acetic acid-induced colitis had higher serum IL-6 levels. This increase has been linked to disease severity in UC patients and has been shown to play a role in pro-inflammatory processes and dysregulation of immune function. TNF-α is a critical mediator in the aetiology of UC and is elevated in many patients, leading to neutrophil infiltration and tissue destruction by higher levels of adhesion molecules[22-24]. The results showed that CPE was able to reduce the MPO, MDA, Proinflammatory mediators and oxidative stress associated with acetic acid-induced colitis. These effects are probably due to anti-inflammatory, antioxidant, and mucosal protective actions.

The presence of flavonoids, and other polyphenolic compounds in the CPE may have contributed to the effects of *Caesalpinia pulcherrima* leaf extract. Presupposing that, flavonoids present in *Caesalpinia pulcherrima* leaf extract would have increased mucosal prostaglandin content, decreasing histamine secretion from mast cells by inhibition of histidine decarboxylase and scavenge free radicals as well as a gastric proton pump, inhibition of the lipoxygenase[25].

Conclusion

According to the results, *Caesalpinia pulcherrima* leaf extract is beneficial against acetic acid-induced colitis in rats, due to its anti-inflammatory, antioxidant and mucosal protective properties.These findings encourage the scientific community to continue researching in this area.

Significance Statement

This study highlights for the first time, the curative effect of *C. pulcherrima* against acetic acid-induced colitis in rats. The plant, therefore, has prospective use for protection from ulcerative colitis of different etiologies, attributed to its ability to boost anti-inflammatory, antioxidant and mucosal protective properties. This study will help the researchers to uncover the different protective properties of the medicinal plants.

Acknowledgement

The authors are thankful to Shri G. Brahmaiah, Shri C. Srinivasa Baba, and Shri M.M. Kondaiah Management of Gokula Krishna College of Pharmacy, Sullurpet, SPSR Nellore Dist, A.P, India and Management of PRRM College of Pharmacy, Kadapa, A.P, India for availing the laboratory facilities during research studies.

Conflicts of Interest:

The authors declare no conflict of interest

Funding

The authors did not receive any grant from any commercial, governmental, or non-profit organizations related to this work.

Disclaimers

The opinions expressed in this article are the author's personal views and do not represent that of their affiliated organizations, employers, or associations.

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