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Evaluation Of Clerodendrum Paniculatum Leaves Extract On Experimental Models Of Inflammatory Bowel Disease

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ABSTRACT :

Herbal drugs are the best treatment for IBD nowadays, which not only treats ulcers but also inhibits the cause of prolonged side effects of synthetic drugs. This study aims to overcome ulcerative colitis caused by oxidative stress. *Clerodendrum paniculatum* alcoholic extract of leaves studied on rodents (150-250g) using acetic acid-induced ulcerative colitis model. The studies include an acute toxicity study (2000mg/kg), treatment with a high dose (400mg/kg), and a low dose (200mg/kg) of *Clerodendrum paniculatum* in comparison to the standard drug Sulfasalazine (500mg/kg). The extract has been studied for its anti-inflammatory and anti-oxidant activity. This study was assessed by body weight and colon weight changes (physical parameter), MPO and LPO (Biochemical parameters), and ulcer scores (macroscopic evaluation). These parameters showed a significant result when compared to the diseased group. The results showed a protective effect of the extract on epithelial cells in the distal colonic portion via pre-treatment. Thus, *Clerodendrum paniculatum* extract 400mg was found to have higher potency ($p< 0.01$) compared to 200mg ($p< 0.05$). The study concludes that the alcoholic extract of *Clerodendrum paniculatum* leaves was effective against inflammatory bowel disease (ulcerative colitis) induced by acetic acid.

Keywords: Acetic acid, Clerodendrum paniculatum, Inflammatory bowel disease, Ulcerative colitis.

INTRODUCTION:

Inflammatory bowel disease (IBD) is a chronic relapsing and remitting condition affecting mainly the gastrointestinal tract. Although it is believed that an abnormal immune response is elicited against the intestinal microbiota in genetically predisposed individuals. The Initial development of IBD is not fully known, crucial elements of etiopathogenesis have been elucidated by research using human biological compounds.[1] It is a common and chronic gastrointestinal disorder distinguished by intestinal inflammation and mucosal damage.[2] The majority of symptoms of IBD include diarrhea, abdominal pain, fatigue, fever, dehydration, vomiting, bleeding stool, loss of appetite, and weight loss. IBD is a spectrum of chronic idiopathic inflammatory intestinal conditions. Conventionally divided into two subtypes: Crohn's Disease (CD) and Ulcerative colitis (UC). The CD is specified by Transmural inflammation at any part of the gastrointestinal tract but more commonly near to ileocecal valve and UC is confluent mucosal inflammation of the colon from the anal verge to extending proximally for variable extent[3] Males and females affect in equal proportions. For both conditions, the etiopathogenesis is multifactorial. Although the cause of IBD is unclear, numerous environmental risks factors such as smoking, appendectomy, infections, antibiotics, diet, and lifestyle and this influence both the initiation and progression of colitis.[4] A major cause for IBD is the presence of microorganisms in the GI tract and loophole. A combination of oxidative stress, abnormal gastrointestinal tract, and immune degradation also causes IBD.[5] The genetic background of IBD with the identification of circa 240 genetic loci associated with IBD thus far (5–7).[1] Many drugs are used in IBD, often symptomatic or temporary relief. Most of the current therapy used in the treatment of IBD is glucocorticosteroids and 5-amino salicylic acid. Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effects. Many drugs are used in IBD, often symptomatic or temporary relief. Most of the current therapy used in the treatment of IBD is glucocorticosteroids and 5-amino salicylic acid. Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effects. Normally herbal plants are free from side effects or adverse effects and they are low-cost medicines[6,,7] there is a need to develop safe and effective alternative therapeutic agents for the treatment of IBD. Considering oxidative stress is one of the major etiopathological factors in IBD, the anti-oxidants could be expected to provide relief and protection[8]. Clerodendrum species are the most spectacular and are reported to have ethnomedicinal importance as the plant was used as a remedy for ailments and disorders are few.[9] The leaves, roots, and flowers are reported for their anti-oxidant, anti-inflammatory, hepatoprotectives, anti-diabetic activities, and so on.

Clerodendrum paniculatum is known to possess phytoconstituents like terpenes, alkaloids, phenolic acid, sterols, flavonoids, tannins, and glycosides. Further, this plant was reported to possess antioxidant, anti-inflammatory, and anti-hemorrhoid effects[10] which may be beneficial in treating IBD. However, no scientific data regarding the activity of *Clerodendrum paniculatum* leaves on IBD was available. Hence the present study was designed to evaluate the protective effect of *Clerodendrum paniculatum* leaves for inflammatory bowel disease.

MATERIALS AND METHODS

Drugs and Chemicals:

Chemicals such as acetic acid and sulfasalazine were of pure analytical grade and procured from a local supplier.

Preparation of alcoholic extract of leaves

The *Clerodendrum paniculatum* leaves were collected from the Dakshina Kannada district and Authenticated by a Botanist. The shade-dried powdered leaves were extracted by the Hot percolation method (Soxhlet Apparatus) using 95% ethanol. The extract was filtered through a cotton plug followed by a Whattman filter paper no.1 and then concentrated to syrupy consistency by using a rotary flash evaporator at low temperature. The dried extract was preserved in an airtight container and kept at 4-5°C until further use.

Preliminary phytochemical screening of alcoholic extract

The alcoholic extract of *Clerodendrum paniculatum* was subjected to preliminary phytochemical screening as per the standard procedure.

Experimental Animals

The Wistar albino rats (150 - 250g) of either sex was used for the experiment. Standard conditions (temperature 22±2°C, relative humidity 50±5%, and 12h light/dark cycle) were maintained for the animals. These animals were irregularly selected as experimental and control groups and housed separately in sanitized polypropylene cages containing sterile paddy husks as bedding. They were free to access a standard pellet diet and water ad libitum. The experimental protocols were reviewed and approved by the Institutional Animals Ethics Committee (Ref. No: SCP/IAEC/F150/P202/2022) before the initiation of the experiment and the care of the laboratory animals was taken as per the CPCSEA regulations.

Acute Toxicity Study

An acute toxicity study of the extract was performed using three female Wistar rats according to OECD guidelines No. 423. The animals fasted overnight and the extract was administered orally with a limiting dose of 2000 mg/kg of animals. Animals were observed continuously for the first 4 hours and monitored for 14 days for any mortality and general behaviour of animals, signs of discomfort, and nervous manifestations.[11]

Acetic Acid-Induced Ulcerative Colitis

Experimental design:

The Wistar albino rats (150-250g) of either sex was selected randomly and divided into five groups of six each. The different groups were assigned as follows.

Group 1: Vehicle control (Saline)
Group 2: IBD control (Acetic acid, 2ml of 4% v/v in 0.9% saline intra-rectally on the 8th day)
Group 3: Reference standard (Sulfasalazine, 500mg/kg, p.o. + Acetic acid, 2ml of 4% v/v in 0.9% saline intra-rectally on 8th day)
Group 4: Test group (*Clerodendrum paniculatum* extract 200mg/kg, p.o. + Acetic acid, 2ml of 4% v/vin 0.9% saline intra-rectally on 8th day)
Group 5: Test group (*Clerodendrum paniculatum* extract 400mg/kg, p.o. + Acetic acid, 2ml of 4% v/vin 0.9% saline intra-rectally on 8th day)

Treatment

Animals fasted overnight with free access to water. Except for group 3 (standard group), all the other groups of animals were pre-treated with respective drugs for 7 days and further treatment was continued for 11 days. Standard drug sulfasalazine was given to group 3 from the day of induction of IBD till the 11th day. On the 8th day, all the animals except group 1 were induced IBD under light ether anesthesia by intra-rectal administration of Acetic acid (2 ml of 4% v/v in 0.9% saline) by inserting an 8 cm long 2.0 mm diameter soft pediatric feeding tube. After acetic acid administration, the animal was held horizontally with a slightly upright position for 2 minutes to prevent spilling of acetic acid. Vehicle control animals were administered with an equal volume of normal saline for 11 days.

Evaluation

On the 11th day, 2 h after respective drug treatment the animals were sacrificed by euthanasia (overdose of Ketamine i.p.) and the colon was dissected. The dissected colon was used to assess the following parameters,

1. **Physical parameter**: Body weight of animal and, weight and length of the colon.
2. **Macroscopic features**: Quantification of inflammation by scoring ulcers.
3. **Biochemical parameter**: Estimation of myeloperoxidase and lipid peroxidation in colon tissue homogenate.

Physical parameter

Physical parameters like animal body weight, colon weight, colon length, and colon weight/length ratio were used to evaluate animal disease status. The animals were weighed each day and the percentage of change in body weight was evaluated.[12]

Macroscopic evaluation

The score for the colon: For each animal, the distal 10cm portion of the colon was removed, cut longitudinally cleaned in saline, and scored for macroscopic features using a scoring pattern.

Biochemical parameters

Myeloperoxidase (MPO) assay:

To measure MPO activity, colonic samples were minced on ice and homogenized in 10 ml of ice-cold 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% HETAB and 10 mM EDTA. The homogenates were then sonicated and centrifuged for 20 min at 12,000 rpm. MPO activity was measured spectrophotometrically as follows: 0.1ml of supernatant was combined with 2.9ml of 50mM phosphate buffer containing 0.167 mg/ml *O*-dianisidine hydrochloride and 0.0005% H_2O_2. The change in absorbance was measured spectrophotometrically at 460nm.
One unit of MPO activity is defined as the change in absorbance per minute at room temperature, in the final reaction.

Calculation of MPO activity
MPO activity (U/g) = X/Weight of the piece of tissue.
Where X=10× Change in absorbance per minute/volume of supernatant taken in the final reaction.[13]

Lipid peroxidation (LPO) assay:
Lipid peroxidation was estimated by taking 2.0ml of the tissue homogenate and mixing it with 2.0ml of freshly prepared 10% w/v TCA then the mixture was allowed to stand in an ice bath for 15 minutes. After centrifugation, 2.0 ml of the clear supernatant solution was mixed with 2.0 ml of freshly prepared thiobarbituric acid. The resulting solution was heated in a boiling water bath for 10 minutes and immediately cooled in an ice bath for 5 minutes. The colour developed was measured at 532nm against a reagent blank. The values were expressed as nmol of MDA/mg protein.[14]

Statistical Analysis:
All data were expressed as Mean ± SEM. The statistical significance between groups was compared using one-way ANOVA, followed by Tukey's multiple comparison tests.

RESULTS

Preliminary phytochemical screening
The preliminary phytochemical test of *Clerodendrum paniculatum* leaves extract is performed and the results showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins and phenols.

Acute Toxicity Study
There was no mortality amongst the dosed groups of animals and did not show any toxicity or behavioural changes and the extract was found to be safe or non-toxic in rats.
The doses of 200 mg/kg and 400mg/kg, p.o. extract of *Clerodendrum paniculatum* leaves was used for the present study.

Acetic acid-induced colitis in albino rats
Effect on body weight
There was a significant decrease in the body weight in the acetic acid-induced animals compared to vehicle control animals. The animal treated with Sulfasalazine ($p< 0.001$) and a high dose of *Clerodendrum paniculatum* ($p<0.01$) significantly restored the body weight whereas a lower dose of *Clerodendrum paniculatum* ($p<0.05$) moderately stored the decreased in body weight as compared to IBD control.

Effect on colon weight measurement
Acetic acid-induced increase in colon weight significantly ($p<0.001$) compared to the vehicle control group. Whereas the drug Pre-treated group *Clerodendrum paniculatum* 200 mg /kg ($p<0.05$), *Clerodendrum paniculatum* 400mg/kg ($p<0.01$), and Sulfasalazine ($p<0.001$) decreased colon weight significantly and dose-dependent compared to IBD control group.

Effect on colon length measurement
The disease induction by instillation of acetic acid intra-rectally causes shrinkage in the colon length in the acetic acid group significantly ($p<0.001$) compared with the vehicle control group, drug-treated group *Clerodendrum paniculatum* 200mg/kg ($p<0.05$), *Clerodendrum paniculatum* 400mg/kg ($p<0.01$), and Sulfasalazine ($p<0.001$).

Effect on macroscopic ulcer scores
Intra-rectal instillation of acetic acid (2ml of 4%v/v) caused an inflammatory reaction in the colon. Evaluation based on macroscopic features showed that ulcer score values significantly increased ($p<0.001$) in the acetic acid-induced group compared to the vehicle control.

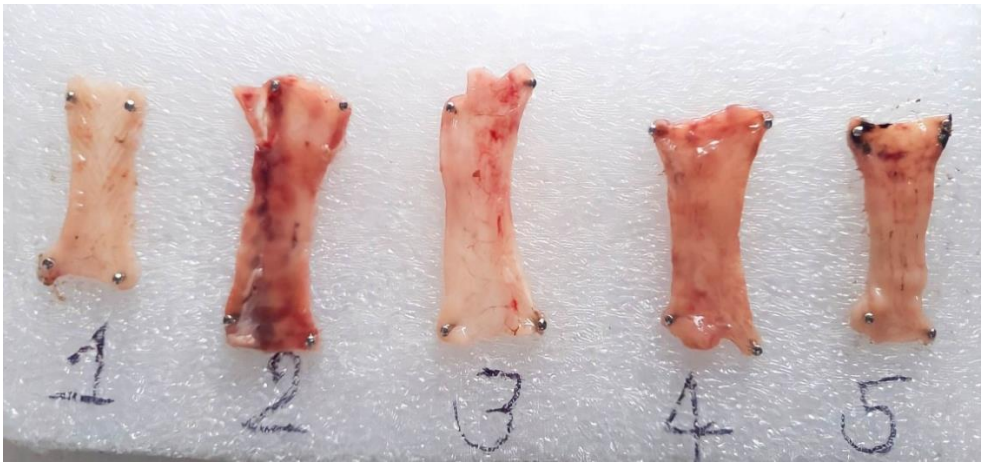

Fig: Vertical sections of the Colon of treated rats

1) Vehicle Control: Shows no ulceration, **2) IBD control (Acetic acid-induced):** Shows high level of ulceration, **3) Sulfasalazine treated:** Shows low ulceration, **4) Extract (200mg/kg):** Shows moderate ulceration, **5) Extract (400mg/kg):** Shows mild ulceration.

The drug treatment with sulfasalazine ($p<0.01$) and a high dose of *Clerodendrum paniculatum* 400mg/kg ($p<0.01$) showed a significant decrease in ulcer score values whereas a lower dose of *Clerodendrum paniculatum* 200mg/kg ($p<0.05$) showed a moderate decrease in ulcer score as compared to IBD control group.

Effect of Biochemical Parameters

Myeloperoxidase activity (U/g)

The MPO assay showed that there is a significant increase ($p<0.001$) in the MPO activity of acetic acid of the induced group compared to the vehicle control group. The drug treatment with sulfasalazine ($p<0.001$) and 400mg/kg of *Clerodendrum paniculatum* ($P<0.01$) showed a significant decrease in MPO activity compared to the acetic acid-induced group.

Lipid peroxidase activity (µ mol/g)

The LPO assay showed that there was a significant increase in LPO activity of the acetic acid-induced group compared to the vehicle control group. The drug treatment with Sulfasalazine ($p<0.001$) and 400mg/kg of *Clerodendrum paniculatum* ($p<0.01$) showed a significant decrease in LPO activity whereas 200mg/kg of *Clerodendrum paniculatum* ($p<0.05$) showed moderate decrease LPO activity as compared to IBD control group.

Table No 1. Effect of *Clerodendrum paniculatum* on body weight in acetic acid-induced colitis

Group	1st day Body weight(g)	11th day Body Weight(g)	Percentage of body weight
Vehicle control	250±0.52	265±0.24	105.87%
Acetic acid	210±0.24###	175±0.51###	83.48%###
Sulfasalazine	250±0.42***	210±0.41***	84.02%***
Extract (200mg/kg)	225±0.34*	198±0.38*	88.03%*
Extract (400mg/kg)	175±0.43**	160±0.25**	91.35%**
All the values are Mean±SEM n=6, One-way ANOVA, followed by Tukey's post-test ###p<0.01 compared to normal group, *p<0.05, **p<0.01, ***p<0.0001 compared to IBD control.			

Table No 2. Effect of *Clerodendrum paniculatum* on physical parameters in acetic acid-induced colitis

Group	Colon weight (g)	Colon length (cm)	Ratio (mg/cm)
Vehicle control	0.9±0.05	7±0.2	125.01
Acetic acid	1.64±0.02###	7±0.17###	228.73###
Sulfasalazine	1.02±0.09***	7±0.2***	141.68***
Extract (200mg/kg)	1.22±0.02*	7±0.39*	165.09*
Extract (400mg/kg)	1.08±0.05**	7±0.22**	149.59**

All the values are Mean±SEM n=6, One-way ANOVA, followed by Tukey's post-test ###p<0.01 compared to normal group, *p<0.05, **p<0.01, ***p<0.0001 compared to IBD control.

Table No 3. Effect of *Clerodendrum paniculatum* on macroscopic ulcer scores in acetic acid-induced colitis

Group	Macroscopic score
Vehicle control	0.00
Acetic acid	4.5±0.5###
Sulfasalazine	2.5±0.4***
Extract (200mg/kg)	3±0.3*
Extract (400mg/kg)	2.25±0.4**
All the values are Mean±SEM n=6, One-way ANOVA, followed by Tukey's post-test ###p<0.01 compared to normal group, *p<0.05, **p<0.01, ***p<0.0001 compared to IBD control.	

Table No 4. Effect of *Clerodendrum paniculatum* on MPO and LPO activity in acetic acid-induced colitis

Group	MPO activity (U/g)	LPO activity (µ mol/g)
Vehicle control	0.850±0.03	0.299±0.03
Acetic acid	3.98±0.01###	0.760±0.01###
Sulfasalazine	1.19±0.03***	0.459±0.02***
Extract (200mg/kg)	2.18±0.01*	0.499±0.01*
Extract (400mg/kg)	1.29±0.02**	0.501±0.02**
All the values are Mean±SEM n=6, One-way ANOVA, followed by Tukey's post-test ###p<0.01 compared to normal group, *p<0.05, **p<0.01, ***p<0.0001 compared to IBD control.		

DISCUSSION

The present study was undertaken to assess the protective effect of the alcoholic extract of *Clerodendrum paniculatum leaves in acetic acid-induced IBD.* LD_{50} studies of the extract were conducted in Wister albino rats by using OECD guidelines number 423. Hence the result of the extract at 2000mg/kg dose confirms it is non-toxic nature of the plant.[15]

The model used for the study of IBD is acetic acid-induced ulcerative colitis. It affects the distal colon portion of rats (150-250g), which shows massive necrosis of mucosal and submucosal layers. The mechanism of inflammation for this model involves the entry of the protonated form of the acid into the epithelium, where it dissociates to liberate protons causing intracellular acidification that most likely accounts for the epithelial injury observed. The inflammation response includes the activation of cyclooxygenase and lipoxygenase pathways and generates inflammatory mediators like prostaglandins and leukotrienes.[16]

Acetic acid installation increases colon weight/length ratio due to shrinkage of the colon, also it plays an important role by increasing the macroscopic scores for inflammation and increasing biochemical parameters like MPO and LPO. Hence this condition is improved via pre-treatment of alcoholic extract of *Clerodendrum paniculatum* at a dose of 200 and 400mg/kg on animals.

The *Clerodendrum paniculatum* extract at 400mg/kg showed better activity compared to 200mg/kg, which shows a decrease in the parameters like macroscopic scores for inflammation and MPO, LPO, the colon weight/length ratio and also showed retained total body weight. The protective action of *Clerodendrum paniculatum* extract was comparable to the standard drug sulfasalazine.

Clerodendrum paniculatum extract contains anti-inflammatory and antioxidant compounds such as flavonoids and other polyphenolic compounds that may constitute an interesting approach in the downregulation of colitis conditions.[17]

CONCLUSION :

From the present investigation, it can be concluded that the alcoholic extract of *Clerodendrum paniculatum* leaves shows potential activity against various pathological changes that occurred during colitis induced by acetic acid due to the presence of active constituents such as flavonoids, alkaloids, saponins, steroids and other phenolic compounds, which possess antioxidant and anti-inflammatory activity.

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