

Research Article**Investigations on *Corchorus aestuans* extracts for ulcerative colitis in experimental animals****Rajeev Ranjan¹, Mohan Lal Kori¹, Santram Lodhi^{2*}**¹Vedica College of B. Pharmacy, RKDF University, Ayodhya bypaas road, Bhopal, M.P. 462033²Sri Sathya Sai Institute of Pharmaceutical Sciences, RKDF University, Ayodhya bypaas road, Bhopal, M.P. 462033

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Abstract

Objective: Present study was aimed to investigate potential of *Corchorus aestuans* for ulcerative colitis in experimental animals. **Material and methods:** Ethanolic extract of *Corchorus aestuans* was (aerial parts) use for phytochemical screening and Dextran Sulphate Sodium (DSS) Induced colitis in rats. The disease activity index and wet colon weight for different groups were observed for assessment of healing effect. Biochemical parameters MPO activity, antioxidant assessment, and MDA level in tissue were determined. **Results:** The disease activity index and weight of colon for colitis control group were found to be 6.25±0.68, 185.36±5.31, respectively. The disease activity index and colon weight for EECA were observed significantly decreased in dose dependent manner. DSS induced colitis significantly elevated MPO activity, whereas administration of EECA strongly inhibited MPO activity in rats with 200mg/kg as well as similar to the standard drug. Treatment with EECA exerted, to some extent, effects on reducing the colonic MDA level compared to animals that received DSS alone. The ethanolic extract of *Corchorus aestuans* (EECA) restored up to the normal level of antioxidant parameters, that was confirmed the potent antioxidant effect of ethanolic extract. **Conclusion:** The present study reveals that the improved condition of ulcerative colitis by *Corchorus aestuans* extract may be due to its capacity to enhance tissue antioxidant levels.

Keywords: *Corchorus aestuans*, ulcerative colitis, Dextran Sulphate Sodium, antioxidant

Introduction

Ulcerative colitis (UC) is a rectal and colonic mucosal chronic, idiopathic, inflammatory bowel disease (IBD). It is characterized by colonic inflammation, most likely due to the infiltration of polymorphonuclear cells, lymphocytes, monocytes and plasma cells, accompanied by oxygen-free radicals, which ultimately leads to mucosal alteration and ulceration (Cho *et al.*, 2007). Abdominal pain and bloody diarrhea are the most common symptoms. But may include; tiredness, weight loss, appetite loss, body fluids, and nutrients (Tai *et al.*, 2007). A combination of blood tests, biopsies, radiography and colonoscopy helps to diagnose UC firmly (Lehne *et al.*, 2004). It is also characterized by the development of intestinal inflammation, most likely resulting from immune system interaction, genetic susceptibility, feeding habits and

drug interactions, ulcerative colitis as a global disease, chronic inflammatory intestinal disease (IBD) affects the rectal and colonic mucosa. The colon, concerned primarily with water resorption, has a flat, glandular surface with no villi at all. Its endothelium is covered by a mucus layer associated with the protection of the epithelial surface against mechanical stress and bacterial pathogens and luminous components such as stomach acidity and proteolytic enzymes (Faure *et al.*, 2003).

Corchorus aestuans is a pantropical species, thought by some to originate from the tropics in Africa and South-East Asia. It was distributed in Northern and Southern America: Mexico, Antigua and Barbuda, Bahamas, Barbados, Cayman Islands, Cuba, Dominican Republic, Grenada, Haiti, Jamaica, Montserrat, Puerto Rico, St. Kitts and Nevis; St. Lucia; St. Vincent and Grenadines; Trinidad and Tobago; Virgin Islands (British), Virgin Islands (U.S.), Guatemala, Nicaragua, Guyana, Venezuela, Brazil, Colombia and Ecuador. It was also found South-East Asia, Mediterranean region and throughout tropical Africa from Senegal eastward to Somalia and southward to South Africa, and it is locally

***Address for Corresponding Author:**

Dr. Santram Lodhi

Sri Sathya Sai Institute of Pharmaceutical Sciences, RKDF University, Ayodhya bypaas road, Bhopal, M.P. 462033

Email: srlodhi78@gmail.com

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cultivated (Khare, 2007; Hyde et al., 2015).

Preliminary phytochemical investigation of various extracts of leaves of *Corchorus aestuans* showed the presence of phenolic compounds, flavonoids, glycosides, carbohydrates, proteins, amino acids, fatty acids, saponins, phytosterols, triterpenoids, cardiac glycosides and tannins (Patel and Patel, 2013; Baskaran et al., 2011). On cooking the leaves discharged a large quantity of mucilage, making them very slimy. The seeds contained 22.6% protein and 8.3–12.8% oil. Amino acids in the seed included valine, lysine, serine, aspartic acid, threonine and phenylalanine (Patel R and Patel, 2013; Khan et al., 2006). The oil contained β -sitosterol and the fatty acids (palmitic acid, stearic acid, oleic acid and linolenic acid). The seed also contained corchorine, a glycoside of the strophanthidine group, and quercetin, a flavonoid. β -sitosterol, lupeol, betulin, 2-methyl anthraquinone, scopoletin and corchoroside-A were isolated from the capsule extract of *Corchorus aestuans* (Huang et al., 1980; Ramadevi and Ganapaty, 2012). The seeds also contained cardenolides, beta-sitosterol, ceryl alcohol, oligosaccharides. Nutritional chemical analysis showed that leaves contained protein 3.7%, β Carotene 76.33 mg/kg, iron 184.07 mg/kg and potassium 4000mg/kg (Choudhary et al., 2013). The fusidic acid together with β -sitosterol, 2-methylanthraquinone and coumarin (scopoletin) were isolated from the leaf extract of *Corchorus aestuans* (Ramadevi and Ganapathy, 2012). The leaves are widely eaten as a vegetable. In northern Benin, for instance, it is consumed as leafy vegetable in a mucilaginous sauce, and its cultivation in the rainy season for household consumption has been recorded in south-western Benin. However, the consumption of *Corchorus aestuans* is lower than that of the cultivated *Corchorus olitorius* L. and *Corchorus tridens* L. In some tribes in Africa there are taboos with regard to *Corchorus aestuans*; its consumption is for instance forbidden in Ouatchi communities in Togo. In north-eastern India the root is cooked as a vegetable. The foliage is browsed by all livestock (Khare, 2007).

Corchorus species are used as diuretic, chronic cystitis, gonorrhoea and dysuria antihistaminic, antiinflammatory, antimicrobial, cardiogenic, and also to increase the viscosity of the seminal fluid. Several important bioactive molecules were reported which includes glycosides, their aglycone and polysaccharides, triterpenoids, phenolics, sterols and fatty acids. Therefore, we aimed to evaluate aerial parts of *Corchorus aestuans* for treatment of colitis on animal model of colitis induced by dextran sulfate sodium (DSS).

Material and methods

Collection and identification of plant material

The aerial parts of *Corchorus aestuans* were collected in the month of March to April around the campus of RKDF University, Bhopal

(M.P.) India. Plant material was identified, dried under shed at room temperature and coarsely powdered moderately and passes through sieve No. 10.

Extraction of plant material

The powdered plant material (500 g) aerial parts of *Corchorus aestuans* were successively extracted in a Soxhlet apparatus with petroleum ether (60-80 °C), Chloroform, Ethyl acetate, ethyl alcohol (95%) and finally with Chloroform water (by maceration process). After each extraction test was performed to see whether the drug had been completely exhausted or not. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent. After ethanol extraction the marc obtained was dried and macerated with chloroform water for 24 hrs repeatedly two to three times. The liquid extracts were collected in a tare conical flask. The solvent removed by distillation. The last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w yield was calculated (Wallis, 1955; Hoover, 1970; Brain and Turner, 1975). All successive solvent extracts such as petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts were subjected for qualitative analysis.

Animal protocol

Wistar albino rats (150-200g) of either sex were selected for the experiment. They were housed individually in well-ventilated, temperature controlled (26±2°C) animal room for seven days of period prior experiment. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and they were kept under standard environmental conditions of laboratory temperature and water *ad libitum*. The animals were maintain alternate cycle of darkness and light at 12 hours. The animals were fasted for at least 12 hours before the onset of experiment. The experimental protocols were approved by Institutional Animal Ethics Committee.

Acute toxicity study

Before exploring any new drug moiety, be a natural or synthetic, its safety studies have to be performed in order to find out the therapeutic window, minimum effective concentration and toxic dose level. This is done to assess that till which concentration, the drug under investigation is safe to be further explored for its therapeutic usefulness. Previously, the Lethal Dose Studies used to be conducted, known as LD50 determinations, in which the dose at which 50% of the cattle die was calculated to estimate the drug's toxicity rate and was a determining factor in the calculation

of the therapeutic dose. The acute oral toxicity study of crude extract of *Corchorus aestuans* was carried out as per OECD guideline 423. As the second alternative to the conventional acute toxicity test, described in Test Guideline 401, the original Guideline 423 was adopted in March 1996.

The ethanolic extract of *Corchorus aestuans* was concentrated under reduced pressure to dryness and then suspended in 0.5% CMC-Na solution for pharmacological evaluation afterwards.

Dextran Sulphate Sodium (DSS) Induced Colitis

The administration of DSS contained in water causes haematochezia, body weight loss, shortening of the intestine, mucosal ulcers and neutrophil infiltration. Acute colitis is regarded to be induced but not obtained by innate immunity. On the other side, the chronic stage is reported to be caused by lymphocytes activated by the cytokines secreted from the activated macrophages (Jurjus *et al.*, 2004).

In rats, ulcerative colitis was caused by adding DSS (Dextran Sulfate Sodium) to water bottles, resulting in a 3% (w / v) solution (Hirata *et al.*, 2001). Free access to water comprising 3 percent oral DSS for 7 days was provided to the cattle. For 7 consecutive days, all treatment regimens were continued. Drugs were administered once daily by oral gavage and suspended in Sodium CMC. Clinical activity results were evaluated on the 8th day and the animals were anesthetized with ether and blood was gathered for biochemical assessment through retro orbital puncture. Daily recorded body weight, consistency of stools and gross bleeding.

Six animals were taken in each group and three groups were made in each wound model. The animals were divided into 5 groups consisting 6 animals in each:

- Group I normal or untreated animals
- Group II is control received Dextran sodium sulfate (3%w/v in drinking water) + 0.9% saline at a dose of 50 ml/kg,
- Group III received Dextran sodium sulfate (3%w/v in drinking water) + ethanolic extract suspension 150 mg/kg
- Group IV received Dextran sodium sulfate (3%w/v in drinking water) + ethanolic extract suspension 200 mg/kg.
- Group V received Dextran sodium sulfate (3%w/v in drinking water) + Sulfasalazine in a dose of 500 mg/kg suspension.

Assessment of colon damage by macroscopic scoring

A clinical score assessing weight loss, stool consistency, and colon bleeding as described by Cooper, divided by 3, quantified the score for disease activity (Niu *et al.*, 2013). Each rating was as follows:

Change in body weight loss: 0: none; 1: 1–5%; 2: 5–10%; 3: 10–20%; 4: >20%;

Stool blood: 0: negative; 1: +; 2: ++; 3: +++; 4: ++++

Stool consistency (0: normal, 1 and 2: loose stool, 3 and 4: diarrhea)

Body weight loss was calculated as the percent difference between the original body weight (day 0) and the body weight on any particular day.

Myeloperoxidase (MPO) assay

MPO activity was identified using an MPO detection kit using the O-dianisidine technique (Liu and Wang, 2011; Yang *et al.*, 2012). Blood was gathered and centrifuged from the eyes. The MPO activity was evaluated at 460 nm by absorbance using a spectrophotometer (Shimadzu). MPO activity was described as the enzyme degrading 1 μ mol per minute at 37 μ C and expressed in units per liter of serum.

Determination of malondialdehyde (MDA) content

By Mihara and Uchiyama (1978), lipid peroxidation was evaluated as the colon's MDA content. In short, MDA's colorimetric determination is based on the response of one reactive aldehyde molecule with two thiobarbituric acid molecules at low pH (2–3) and 45 min at a temperature of 95°C. By treatment with N-butanol obtained the resulting purple color and spectrophotometrically determined the absorbance at 532 and 520 nm. As a measure of colonic MDA content, the distinction in optical density between the two wavelengths was used. MDA's final value was depicted as protein nmol/mg.

Colon homogenate (0.5 ml) and 1 ml of 0.15 M KCl were added to test tubes. Peroxidation was initiated by adding 100 μ l ferric chloride after incubation at 37°C. The reaction was stopped by adding 2 ml of ice cold mixture of 0.25 N hydrochloric acid containing 15% trichloroacetic acid, 0.30% TBAR and 0.05% BHT and reaction mixture heated for 1 h at 80°C. The sample was cooled and centrifuged. The absorbance of supernatant was measured at 532 nm. Results were expressed in nmol of MDA/mg protein.

Determination of antioxidants assay

Tissue sample of colon was used for antioxidant assay. Catalase was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in granuloma tissue by the method of Moron *et al.*, (1979).

Statistical analysis

The data were expressed as mean standard deviation (SD). The statistical significance of the difference in each

parameter among the groups was evaluated using one-way analysis of variance (ANOVA) followed by the followed by the multiple comparison test of Tukey–Kramer tests. Criterion for statistically significant difference was chosen to be at $P < 0.01$.

Results and discussion

Ulcerative colitis (UC) is a chronic, idiopathic, inflammatory bowel disease (IBD) of the rectal and colonic mucosa. It is characterized by colonic inflammation, resulting most probably from the infiltration of polymorphonuclear cells, lymphocytes, monocytes, and plasma cells, accompanied by the overproduction of oxygen free radicals, ultimately leading to mucosal alteration and ulceration (Cho *et al.*, 2007).

Aerial parts of *Corchorus aestuans* were collected and identified. The powdered materials of both plants were successively extracted with petroleum ether, chloroform, ethyl acetate, ethyl alcohol and water. The percent yields of each extract were calculated. The percent yields of all extracts of *Corchorus aestuans* found as 6.25% w/w (petroleum ether), 4.6%w/w (chloroform), 3.8%w/w (ethyl acetate), 8.65%w/w (ethyl alcohol) and 4.28%w/w (chloroform water). The TLC of ethanol extracts of *Corchorus aestuans* was done in different solvent systems and spots as well as best separation was observed.

The phytochemical analyses of all extracts were performed qualitatively for different phytoconstituents. *Corchorus aestuans* give positive test of steroids in petroleum ether and

alkaloids in chloroform extract. The ethyl acetate and ethanol extract contains flavonoids, tannins, glycosides. Aqueous extract were found presence of carbohydrates, saponins and amino acids.

Acute toxicity study

Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases no one death was observed within first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and motor activity and behavioral pattern. Attention was also given to observation of tremors and convulsions. We have selected one tenth dose of highest toxic dose level.

Dextran Sulphate Sodium (DSS) Induced Colitis

DSS produces severe macroscopic edematous inflammation in the colon. The disease activity index and wet colon weight for different groups were observed. The disease activity index and weight of colon for colitis control group were found to be 6.25 ± 0.68 , 185.36 ± 5.31 , respectively. The disease activity index and colon weight for EECA were observed significantly decreased in dose dependent manner. EECA 200 mg/kg dose decreases disease activity index and weight of colon significantly as 2.51 ± 0.35 (59.84) and 138.82 ± 4.20 , respectively (Table 1).

Table 1. Effect of ethanolic extract of *Corchorus aestuans* (EECA) on colon of rats

Groups	Disease activity Index (% protection)	Weight of colon (mg/cm)
Normal control	0	136.28 ± 3.54
Control (0.9% saline)	6.25 ± 0.68	185.36 ± 5.31
EECA 150 mg/kg	4.24 ± 0.17 (32.16)	$152.42 \pm 4.37^*$
EECA 200 mg/kg	2.51 ± 0.35 (59.84)*	$138.82 \pm 4.20^*$
Sulfasalazine (500mg/kg)	2.64 ± 0.13 (57.76)*	$137.55 \pm 4.22^*$

n = 6 albino rats per group, value represents Mean S.D. * $P < 0.01$, when compared each treated group with control group.

Table 2. Effect of ethanolic extract of *Corchorus aestuans* (EECA) on MPO and MDA level of colonic tissues of rats

Treatment groups	MPO (OD/g tissue)	MDA (OD/g tissue)
Normal control	33.25 ± 1.67	18.42 ± 0.24
Control (0.9% saline)	56.12 ± 2.30	42.38 ± 0.67
EECA 150 mg/kg	41.62 ± 1.95	28.71 ± 0.46
EECA 200 mg/kg	$32.85 \pm 1.28^*$	$19.82 \pm 0.52^*$
Sulfasalazine (500mg/kg)	$34.27 \pm 1.49^*$	$20.47 \pm 0.85^*$

Values are presented as mean of optical density (OD) \pm SD, * $P < 0.05$, represent significant value compared with control group

Table 3. Effect of ethanolic extract of *Corchorus aestuans* (EECA) on antioxidants level of colonic tissues in rats

Groups	Antioxidants level		
	SOD($\mu\text{g}/50 \text{ mg tissue}$)	CAT($\mu\text{mol}/50 \text{ mg tissue}$)	GSH($\mu\text{mol}/50 \text{ mg tissue}$)
Normal control	37.25 \pm 2.61	15.82 \pm 1.07	42.08 \pm 2.46
Control (0.9% saline)	14.64 \pm 1.52	7.62 \pm 0.81	22.71 \pm 1.85
EECA 150 mg/kg)	27.32 \pm 1.86	11.58 \pm 0.94*	36.88 \pm 1.64*
EECA (200 mg/kg)	36.29 \pm 2.08*	15.29 \pm 0.48*	42.99 \pm 1.88*
Sulfasalazine (500mg/kg)	35.24 \pm 2.46*	15.04 \pm 0.88*	41.28 \pm 2.34*

n = 6 albino rats per group, value represents Mean S.D. *P < 0.01, when compared each treated group with control group

However, the EECA 200 mg/kg showed better results in these parameters, indicating its potent activity at the dose tested. These data were also comparable to the standard drug treatment group.

The effect of ethanolic extract of *Corchorus aestuans* (EECA) on different biochemical parameters were also observed in dose dependent manner. In the experiment, we found that MPO activity was correlated with the development of colonic inflammation. DSS induced colitis significantly elevated MPO activity, whereas administration of EECA strongly inhibited MPO activity in rats with 200mg/kg as well as similar to the standard drug (Table 2). The results of MDA level in tissue also indicated that colonic content of MDA decreased significantly and similar to the standard drug when compared to the DSS model group. Treatment with EECA exerted, to some extent, effects on reducing the colonic MDA level compared to animals that received DSS alone.

The effect of EECA on the various antioxidant level (SOD, CAT and GSH) were observed in table 3. The ethanolic extract of *Corchorus aestuans* (EECA) restored up to the normal level of antioxidant parameters, that was confirmed the potent antioxidant effect of ethanolic extract.

The level of antioxidants in colon tissues were observed significant decrease in colitis control group, may be due increasing free radicles generation. This decreasing level of SOD, CAT and GSH was slightly increased in treatment group with 150mg/kg dose of EECA. But a significant improvement in level of SOD, CAT and GSH was found in treatment group of 200mg/kg dose of EECA as well as standard drug treated group, when compared to colitis control group (Figure 4).

Phytochemical study revealed that flavonoids and phenolic compounds are present in ethanolic extracts of *Corchorus aestuans* (EECA). The free radical scavenging property of these flavonoids plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo*

antioxidant activity. Hence, the probable mechanism of healing of ulcerative colitis by ethanolic extract of *Corchorus aestuans* (EECA) may be attributed to antioxidant and free radical scavenging property, while free radical scavenging activity may be attributed to flavonoids and phenolic compounds.

In conclusion, the observation and results obtained in present study indicated that the antioxidants play a vital role in ulcer healing. These antioxidants are known to quench the superoxide radical and thus prevent the damage of cells caused by free radicals. Thus the present study reveals that one of the mechanisms of the improved condition of ulcerative colitis by *Corchorus aestuans* extract may be due to its capacity to enhance tissue antioxidant levels. These findings could justify, at least partially, the inclusion of this plant in the management of colitis in folk medicine. This study also confirms the promising healing activity of ethnolic extract and deserve for detailed experiments on cellular level and clinical studies in future.

Conflict of interest: None

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