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Preliminary Pharmacological Screening of *Citrullus colocynthis* Linn. Fruits Extracts Using Dextran Sodium Sulfate Induced Colitis in Experimental Animals

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Abstract

The objective of present study was study of preliminary pharmacological screening of *Citrullus colocynthis* fruits extracts for ulcerative colitis using Dextran Sodium Sulfate (DSS)-induced colitis in experimental animal. Different extracts i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *Citrullus colocynthis* Linn. fruits were screened for protective effect in Dextran Sodium Sulfate (DSS)-induced colitis through assessment of disease activity index (DAI), myeloperoxidase (MPO) and malondialdehyde (MDA) levels and antioxidants assay in colonic tissues. Results of study were showed that *Citrullus colocynthis* Linn. give positive test of steroids in petroleum ether and alkaloids in chloroform extract. The ethyl acetate and ethanol extract contains flavonoids, tannins, glycosides and amino acids. Aqueous extract was found presence of carbohydrates, saponins and amino acids. The disease activity index and colon weight were observed significantly decreased after treatment with ethanol extract of *Citrullus colocynthis* (EECC). Other extracts of *Citrullus colocynthis* fruits e.g. CECC, EACC and AECC at the dose of 200 mg/kg were does not showed significant Disease activity Index as well as % protection. Treatment with EECC significantly reduced MPO to 12.54 ± 0.12 and MDA to 44.20 ± 1.64 ($P<0.05$), bringing the values close to normal and comparable to sulfasalazine (MPO: 15.34 ± 0.54; MDA: 45.02 ± 1.73). EECC produced significant improvement in all measured parameters, demonstrating strong anti-inflammatory and antioxidant activities comparable to the standard drug sulfasalazine. This pharmacological screening of different extracts of *Citrullus colocynthis* fruits suggests that ethanol extract mitigates colonic damage through antioxidant replenishment, reduction of myeloperoxidase (MPO) and malondialdehyde (MDA) levels, and mitigating oxidative stress. Among the tested doses, 200 mg/kg of different extracts, the ethanol extract of *Citrullus colocynthis* Linn. fruits produced the most pronounced effects and demonstrated efficacy comparable to the standard drug sulfasalazine.

Keywords: *Citrullus colocynthis*, ulcerative colitis, Dextran Sodium Sulfate, disease activity index, myeloperoxidase, malondialdehyde.

INTRODUCTION

Ulcerative colitis (UC) is one of the major types of inflammatory bowel disease (IBD). It mainly affects the lower end of the digestive system, the large intestine, and the rectum (Ungaro *et al*., 2017. Patients with UC display various clinical symptoms, mainly including abdominal pain, bloody diarrhea, malnutrition, fecal urgency, and tenesmus, while the progression of UC may also cause clinical manifestations in other organs (Feuerstein *et al*., 2019). The development of UC may promote multiple pathological changes in the intestine, such as hyperemic and granular mucosa, petechial hemorrhages, and the formation of ulcers. These alterations are accompanied by multiple histological changes, such as crypt abscess and inflammatory cell infiltration to the lamina propria (DeRoche *et al*., 2014). An increasing body of evidence showed that the pathogenesis of UC is associated with the interplay of multiple factors from host genetics, immunity, microbes, and the environment (Porter *et al*., 2020).

Treatment in UC is directed towards inducing and maintaining remission of symptoms and mucosal inflammation. The choice of the therapeutic method depends on both the extent of colonic involvement and

the severity of the disease at presentation, which are the key parameters to be assessed for the most appropriate treatment during the whole disease course. For years the therapeutic repertoire for UC included amino salicylates, corticosteroids and immune modulators. Recent advances in the understanding of the patho physiological conditions of IBD have provided new immune system modulators as therapeutic tools. Tumour necrosis factor (TNF)-α has been shown to play a central role in the pathogenesis of mucosal inflammation not only in CD, but in UC as well, the blockade of which with the chimeric monoclonal antibody infliximab has shown its efficacy in various published studies (Lehne *et al*., 2010).

The plant species *Citrullus colocynthis* (L.) Schrad (family *Cucurbitaceae*) is common in India and the southern islands (Upadhyay *et al*, 2007). It is commonly known as Colocynth/Bitter apple in English, Indrayan in Hindi, Anedri in Sanskrit, Rakhal in Bengali, and Pcitummatti in Tamil (Devendra *et al*, 2009 and Amamou *et al*, 2011). Its fruit is noted for its cathartic effects and is useful in addressing biliousness, fever, constipation and intestinal parasites. The root is used in jaundice, ascites, rheumatism and urinary diseases. In severe cases of ascites, jaundice, and a number of uterine disorders, including amenorrhea, this medicine is often used as a purgative by doctors (Chopra and Chopra, 2006). Worldwide Leprosy, jaundice, constipation, diabetes, asthma, cancer, bronchitis, joint pain, and mastitis are a few of the conditions for which *Citrullus colocynthis* has been used medicinally (Chopra *et al*, 1958; Sharma, 1998; Abo *et al*, 2008; Sultan *et al*, 2010). *Citrullus colocynthis* fruits possess strong antidiabetic (Jayaraman *et al*., 2009), antioxidant, and anti-inflammatory properties, driven by bioactive cucurbitacins and flavonoids (Hediat *et al*., 2012). Extracts demonstrate potent anticancer activity by triggering apoptosis, alongside antimicrobial, antihypertensive, and powerful purgative effects.

The present study was aimed to perform preliminary pharmacological screening of different extracts i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *Citrullus colocynthis* Linn. fruits for ulcerative colitis using Dextran Sodium Sulfate (DSS)-induced colitis.

MATERIALS AND METHODS

Collection and authentication of plant materials

The *Citrullus colocynthis* Linn. fruits was collected in the month of February to March from the campus of RKDF University Bhopal (M.P.). Plant material was identified and authenticated in the Department of Botany, Department of Botany, Saifia College of Science Bhopal (M.P.). The plant materials were dried in shade, powdered moderately and pass through sieve No. 10.

Extraction and phytochemical screening of different extracts

The powdered plant material (100 g) of *Citrullus colocynthis* Linn. fruits were successively extracted in a soxhlet apparatus with petroleum ether (60-80°C), chloroform, ethyl acetate, ethyl alcohol (95%) and finally with water (by maceration process). All extracts were subjected to phytochemical screening using different chemical test.

Preparation and administration of test samples

The different extracts of *Citrullus colocynthis* were concentrated under reduced pressure to dryness and then suspended in 0.5% CMC-Na solution for pharmacological evaluation afterwards.

Preliminary pharmacological screening of different extracts

Experimental Animals

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 Unilever 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, Guru Ramdas Khalsa Institute of Science and Technology, Jabalpur (Reg. No. GRKIST-P/IAEC(M2)2024/03) as per CPCSEA guidelines for experimental animals.

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. The acute oral toxicity study of crude extract of *Citrullus colocynthis* Linn. was carried out as per the guidelines set by Organization for Economic Co–operation and Development (OECD) guideline 423.

Animal protocol

The animals were divided into eight groups containing 6 animals in each. Various extracts of *Citrullus colocynthis* fruits were given to the respective groups as mentioned below:

Table 1: Experimental animal protocol for study of *Citrullus colocynthis* fruits extracts in Dextran sodium sulfate induced Colitis

Group I	normal or untreated animals
Group II	was control group received Dextran sodium sulfate (3%w/v in drinking water) + Normal saline at a dose of 50 ml/kg
Group III	received Dextran sodium sulfate (3%w/v in drinking water) + petroleum ether extract of *Citrullus colocynthis* suspension (PECC) with dose of 200 mg/kg
Group IV	received Dextran Sodium Sulfate (3%w/v in drinking water) + Chloroform extract of *Citrullus colocynthis* suspension (CECC) with dose of 200 mg/kg
Group V	received Dextran sodium sulfate (3%w/v in drinking water) + Ethyl acetate extract of *Citrullus colocynthis* suspension (EACC) with dose of 200 mg/kg
Group VI	received Dextran sodium sulfate (3%w/v in drinking water) + Ethanol extract of *Citrullus colocynthis* suspension (EECC) with dose of 200 mg/kg.
Group VII	received Dextran sodium sulfate (3%w/v in drinking water) + Aqueous extract of *Citrullus colocynthis* suspension (AECC) with dose of 200 mg/kg.
Group VIII	received Dextran sodium sulfate (3%w/v in drinking water) + Sulfasalazine in a dose of 500 mg/kg suspension

Methods for induction of 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 (Kim *et al*., 2012; Zhou *et al*., 2023). 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 of all animals were recorded on the 8th day for the colonic observation with biochemical assessment of the colon tissue. Observations of each group of animals were recorded daily for body weight, consistency of stools and gross bleeding.

Assessment of colon damage by macroscopic scoring

Quantification of disease activity index (DAI) was performed by scoring data for body weight, stool consistency and gross bleeding in colon. Changes in the body weight were calculated as the percent difference between body weight at day zero and after induction of colitis (Cooper *et al*., 1993).

Each rating of body weight was as follows: 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.

Table 2: Macroscopic observations of animals after treatment with various extracts of *Citrullus colocynthis* fruits on Dextran Sodium Sulfate induced colitis

Groups	Disease activity Index (% protection)	Weight of colon (mg)
Normal control	0	112.34±5.21
Disease Control	8.17±0.087	217.26±7.62
PECC 200 mg/kg	4.52±0.063 (44.67)	163.41±7.14
CECC 200 mg/kg	6.47±0.019 (20.80)	201.08±6.88
EACC 200 mg/kg	5.46±0.064 (33.17)	173.76±6.12
EECC 200 mg/kg	2.17±0.025 (73.43)*	118.42±5.42*
AECC 200 mg/kg	5.51±0.075 (32.55)	178.18±6.74
Sulfasalazine (500mg/kg)	3.10±0.028(62.05)*	122.24±5.64*

n = 6 albino rats per group, value represents Mean ± S.D. *P< 0.05, when compared each treated group with control group.

Estimation of Myeloperoxidase (MPO) assay

MPO activity was identified using an MPO detection kit using the O-dianisidine technique (Chassaing *et al*., 2014). Briefly 10% of tissue homogenate in 0.5% hexa-1,6-bisdecyltrimethyl ammonium bromide (HTAB) in potassium phosphate buffer was sonicated, followed by 3 cycles of freeze and thaw using deep freezer (-20 °C) and again samples were sonicated followed by centrifugation at 4000 rpm for 15 min at 4°C. To 0.1 ml of supernatant, 2.9 ml of potassium

phosphate buffer containing 0.167 mg/ml O-dianisidine HCL was added. The reaction was started by adding 0.0005% hydrogen peroxide. The change in absorbance was recorded at 460 nm for 3 min using a spectrophotometer (Shimadzu). The results were shown as nM of H_2O_2 degraded/min/mg protein.

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.

Table 3: Effect of various extracts of *Citrullus colocynthis* fruits on MPO and MDA level of colonic tissues of DSS induced colitis

Treatment groups	MPO (OD/g tissue)	MDA (OD/g tissue)
Normal control	11.28±0.04	42.15±1.86
Disease Control	37.26±1.05	74.81±3.14
PECC 200 mg/kg	27.57±0.84	62.54±2.85
CECC 200 mg/kg	28.14±1.06	65.21±3.56
EACC 200 mg/kg	21.24±0.75	55.26±2.75
EECC 200 mg/kg	12.54±0.12*	44.20±1.64*
AECC 200 mg/kg	24.50±0.61	58.47±2.14
Sulfasalazine (500mg/kg)	15.34±0.54*	45.02±1.73*

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

Antioxidants assay in colon tissues

Animals from each group were sacrificed by cervical dislocation under thiopental sodium as anesthesia and colon tissues were collected and processed according to the protocol requirement 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 colon tissue by the method of Moron *et al*, (1979).

Table 4: Effect of various extracts of *Citrullus colocynthis* fruits on antioxidants level of colonic tissues in rats

Groups	Antioxidants level		
	SOD (µg/50 mg tissue)	CAT (µmol/50 mg tissue)	GSH (µmol/50 mg tissue)
Normal control	43.26±1.94	36.08±1.64	61.27±2.75
Disease Control	27.43±0.76	22.74±0.75	37.15±1.49
PECC 200 mg/kg	31.85±1.34	24.63±1.06	40.26±1.84
CECC 200 mg/kg	32.45±1.28	25.14±1.18	41.20±1.68
EACC 200 mg/kg	37.71±1.46	29.42±1.64	50.60±2.07
EECC 200 mg/kg	42.18±1.75*	35.16±1.74*	58.14±2.64*
AECC 200 mg/kg	35.42±1.62	28.41±1.26	47.30±1.75
Sulfasalazine (500mg/kg)	40.49±2.04*	32.75±1.51*	55.18±2.16*

n = 6 albino rats per group, value represents Mean ± S.D. *P< 0.05, when compared each treated group with control group

Statistical Analysis

All data were expressed as mean ± SD. The significance of differences between treated groups was determined using one-way analysis of variance (ANOVA) followed by Dunnets' post hoc test. P< 0.05 were considered significant. Statistical Package for Social Scientist (SPSS) Version 22.0 software was used for all statistical analysis.

RESULTS AND DISCUSSION

Phytochemical Study

Citrullus colocynthis Linn. give positive test of steroids in petroleum ether and alkaloids in chloroform extract. The ethyl acetate and ethanol extract contains flavonoids, tannins, glycosides and amino acids. Aqueous extract was 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 doses given to the animals, 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 were noted. Attention was also given to observation of tremors and convulsions. After all observations, we have selected one tenth dose of highest toxic dose level.

Effect of various extracts on Dextran Sulphate Sodium (DSS) Induced Colitis

DSS produces severe macroscopic edematous inflammation in the colon of experimental animals. The disease activity index and wet colon weight of different animal groups were observed.

Animals after treatment with various extracts of *Citrullus colocynthis* fruits, the disease activity index and weight of colon for colitis control group were found to be 8.17±0.087, and 217.26±7.62, respectively. The disease activity index and colon weight for EECC were observed significantly decreased. EECC at 200 mg/kg dose decreases disease activity index and weight of colon significantly as 2.17±0.025 (73.43%) and 118.42±5.42, respectively (Table 2). The standard group of treatment, Sulfasalazine (500mg/kg) was showed disease activity index (along with percentage protection) and weight of colon as 3.10±0.028 (62.05%) and 122.24±5.64, respectively. Other extracts of *Citrullus colocynthis* fruits e.g. CECC, EACC and AECC at the dose of 200 mg/kg were does not showed significant Disease activity Index as well as % protection. PECC extract was showed good Disease activity Index 4.52±0.063 (44.67) but higher than the EECC at dose of 200 mg/kg. The EECE extract at 200 mg/kg showed best results and comparable effect to the standard Sulfasalazine in these parameters, indicating its potent activity at the dose tested.

The results was clearly demonstrate the protective effect of *Citrullus colocynthis* against experimentally induced ulcerative colitis using the DSS model. In the case of *Citrullus colocynthis*, administration of DSS resulted in a marked increase in Disease Activity Index (DAI), with the control group showing a value of 8.17 ± 0.087 compared to zero in the normal group, indicating severe clinical symptoms such as diarrhea, rectal bleeding, and weight loss. Similarly, colon weight was significantly elevated in the control group (217.26 ± 7.62 mg) compared to normal animals (112.34 ± 5.21 mg), reflecting inflammation and edema. Treatment with different extracts produced varying degrees of protection; however, the ethanolic extract (EECC) exhibited the most pronounced effect, reducing the DAI to 2.17 ± 0.025 with 73.43% protection and restoring colon weight to 118.42 ± 5.42 mg, which was very close to normal values and statistically significant ($P<0.05$). Other extracts such as PECC, CECC, EACC, and AECC showed moderate to mild improvements, with DAI values of 4.52 ± 0.063, 6.47 ± 0.019, 5.46 ± 0.064, and 5.51 ± 0.075 respectively. The standard drug Sulfasalazine also significantly reduced DAI to 3.10 ± 0.028 (62.05% protection) and colon weight to 122.24 ± 5.64 mg, indicating that the ethanolic extract was comparable or even superior in efficacy.

Biochemical parameters further substantiated these findings. In *Citrullus colocynthis*, DSS treatment caused a substantial increase in myeloperoxidase (MPO) levels (37.26 ± 1.05) compared to normal animals (11.28 ± 0.04), indicating enhanced neutrophil infiltration, along with a marked rise in malondialdehyde (MDA) levels (74.81 ± 3.14 vs. 42.15 ± 1.86), reflecting increased lipid peroxidation (Table 3). Treatment with EECC significantly reduced MPO to 12.54 ± 0.12 and MDA to 44.20 ± 1.64 ($P < 0.05$), bringing the values close to normal and comparable to sulfasalazine (MPO: 15.34 ± 0.54; MDA: 45.02 ± 1.73). Other extracts showed partial reductions but were less effective than the ethanolic extract.

The antioxidant enzyme profile further confirmed the protective role of the extracts. In *Citrullus colocynthis*, DSS significantly reduced superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels in the control group (27.43 ± 0.76, 22.74 ± 0.75, and 37.15 ± 1.49 respectively) compared to the normal group (43.26 ± 1.94, 36.08 ± 1.64, and 61.27 ± 2.75). Treatment with EECC restored these levels to 42.18 ± 1.75, 35.16 ± 1.74, and 58.14 ± 2.64 respectively ($P<0.05$), indicating near normalization of antioxidant defense, comparable to sulfasalazine-treated animals (Table 4).

Overall, the results across all tables consistently indicate that DSS-induced colitis led to severe clinical symptoms, increased inflammatory and oxidative stress markers and depletion of antioxidant defenses, whereas treatment with plant extracts, particularly the ethanolic extract (EECC), produced significant improvement in all measured parameters, demonstrating strong anti-inflammatory and antioxidant activities comparable to the standard drug.

CONCLUSION

The results of present study demonstrated that ethanolic extracts of Citrullus colocynthis fruits possess significant anti-ulcerative colitis in DSS-induced ulcerative colitis models. The protective effects were evidenced by reduction of disease activity index, decrease in colon weight, suppression of MPO and MDA levels and restoration of antioxidant enzymes. The observed pharmacological activity may be attributed to the presence of flavonoids and glycosides. These compounds are known to possess antioxidant, anti-inflammatory, and free-radical scavenging properties, which collectively contribute to protection against colonic inflammation. Among the tested doses, 200 mg/kg of different extracts, the ethanol extract of *Citrullus colocynthis* Linn. fruits produced the most pronounced effects and demonstrated efficacy comparable to the standard drug sulfasalazine.

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Authors Contributions

Mr. Deependra Singh was performed experimental work and make draft of manuscript. Dr. Santram Lodhi formatted and edited language of the manuscript and approve for final submission.

Conflict of Interest: None

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