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EVALUATION OF AMELIORATIVE EFFECT OF *SYZYGIUM CUMINI* LEAVES ON ULCERATIVE COLITIS

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Inflammatory bowel disease, Ulcerative colitis, Acetic acid, *Syzygium cumini*

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ABSTRACT: Ulcerative colitis is a subtype of Inflammatory Bowel Disease. The leaves of *Syzygium cumini* belonging to the family Myrtaceae were selected to evaluate its efficacy in ameliorating the acetic acid-induced Ulcerative colitis. Male albino Wistar rats were randomly divided into five groups. Group 1 was given the vehicle tween 80, group II received 2 ml of 4% acetic acid solution on 8th day intrarectally, group III was given 2 ml of 4% acetic acid solution once intrarectally on 8th day and 2mg/kg of prednisolone orally for 3 days starting from the day of acetic acid treatment. Group IV and V received 7 days pretreatment with 250 mg/kg and 500 mg/kg of ethanolic leaf extract of *Syzygium cumini* (SCEL), respectively, and 2 ml of 4% acetic acid solution once intrarectally on 8th day. Drug treatment was continued till 10th day. The rats were sacrificed on the 11th day, and the hematological, macroscopical and biochemical parameters were assessed. Pre-treatment with the extract improved the hematological parameters, body weight, and the stool consistency score as compared to the positive control.

INTRODUCTION: Medicinal plants provide medicine to maintain health, prevent disease, and cure ailments. Throughout the world, medicinal plants are used in traditional systems of medicines. In modern drug development, herbal plants and their derivatives have a very important role. Medicinal plants are natural resources in developing new drugs. Inflammatory bowel disease (IBD) is a common chronic inflammatory disease of the gastrointestinal tract. There are two main subtypes of IBD; Crohn's disease (CD) and Ulcerative colitis (UC). The major symptom of UC is inflammation of the mucosal lining of the colon, which results from the interaction between different molecular constituents of the cells.

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This disease is characterized by abdominal pain and diarrhea mixed with blood along with weight loss, fever, and anemia [1, 2]. UC is associated with ulceration, bleeding, and morphological changes in the intestinal mucosa, involving infiltration of polymorphonuclear cells, abscess formation in mucosal crypts and glands distortion. These changes are concentrated in the mucosa and restricted to the colon and rectum [3].

UC increases the possibility for colon cancer caused by the repeated cycle of inflammation that leads to spontaneous mutation in the DNA repair mechanism, oncogenes, and tumor suppressor genes like P 53. Change in the constituent, number, and activity of the colon microflora also contributes to the development of UC [1, 4]. The specific pathogenesis underlying inflammatory bowel disease is complex. Animal models are essential to intrude into mechanistic details that will facilitate better preclinical drug/therapy design to target specific components involved in the disease pathogenesis.

A UC-like phenotype can be induced in animals easily using either chemical administration or bacterial infection. The chemicals used for inducing colitis are DSS, TNBS, oxazolone, acetic acid, and sulfhydryl inhibitors.

Syzygium cumini Linn. (Synonym: *Eugenia jambolan* Linn.) is a very large evergreen tropical tree belonging to the family *Myrtaceae.* The synonym of the plant is black plum or jambolan which is also named as jamun. Jamun is a native of India and East Indies. It is found throughout India up to an altitude of 1800 meters [5]. Different parts of the jambolan were reported for its antioxidant, anti-inflammatory, neuropsycho-pharmacological, anti-microbial, antibacterial, anti-HIV, antileishmanial, antifungal, nitric oxide scavenging, free radical scavenging, antidiarrheal, antifertility, anorexigenic, gastroprotective, antiulcerogenic and radio-protective activities [6, 7].

This study was performed with an objective to evaluate the efficacy of *Syzygium cumini* leaves to ameliorate Ulcerative colitis.

MATERIALS AND METHODS: All chemicals and reagents used were of analytical grade and were procured from Sigma, Ranbaxy fine chemicals, New Delhi, Hi media Mumbai, and NICE Chemicals Ltd, Cochin, Kerala, India. The chemicals used were Pentobarbitone sodium, 4% acetic acid solution, Prednisolone, Ketamine hydrochloride, EDTA, and Turk's fluid. The plant material used was *Syzygium cumini* (L.) Skeels–Leaf, **Fig. 1**.

The plant *Syzygium cumini* was authenticated by Dr. Sr. Tessy Joseph, HOD, Department of Botany, Nirmala College, Muvattupuzha. A voucher specimen is kept in the herbarium of Nirmala College, Muvattupuzha. The voucher specimen number is NCH/2014/NCP/3811.

The leaves were collected in April from hilly areas of Idukki district. The leaves were shade dried and powdered to get coarse powder for extraction. It was stored in polythene bags at room temperature.

Animals: Albino Wistar rats

The Test Sample: Ethanolic extract of leaves of *Syzygium cumini* (SCEL).

Prior to conducting the animal studies approval was obtained from the Institutional Animal Ethical Committee (IAEC No: KMCRET/Ph.D/13/2017-18 and CPCSEA No: 685/PO/02/a/CPCSEA). All the animals were treated according to the guidelines provided by the Institutional Animal Ethical Committee.

FIG. 1: ***SYZYGIUM CUMINI***

Acute Toxicity Study: Acute toxicity study for the ethanolic leaf extract of *Syzygium cumini* extract was carried out in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines 423. Female albino Wistar rats weighing between 150-200 gm were used. The animals received a total ethanolic extract of *Syzygium cumini* orally.

The animals were fasted overnight prior to administration of test substance. The test substance was administered in a single dose of 2000 mg/kg. Food was withheld for 2 h. Animals were observed individually during the first 30 min with special attention during the first four hours, periodically during the first 24 h and daily thereafter for 14 days for general behavior and mortality of the animals. Changes in the body weight and food consumption of the animals were also observed.

Study of Ameliorative Effect of Extracts on Ulcerative Colitis:

Method of Experiment: Acetic acid-induced ulcerative colitis in rats [8, 9.]

Male albino Wistar rats with bodyweight between 150-200 gm were used. Animals were fasted overnight. Water was given *ad libitum.* Group 1 was given the vehicle tween 80, group II received 2 ml of 4% acetic acid solution on 8th day intrarectally, group III was given 2 ml of 4% acetic

acid solution once intrarectally on 8th day and 2mg/kg of prednisolone orally for 3 days starting from the day of acetic acid treatment. Group IV and V received 7 days pretreatment with 250 mg/kg and 500 mg/kg of SCEL, respectively, and 2 ml of 4% acetic acid solution once intrarectally on the 8th day. Drug treatment was continued till 10th day.

Effect of Treatment on Body Weight: The body weight of each rat was taken before and after the treatment.

Effect of Treatment on Stool Consistency: Stool consistency was measured, and scoring was done 24 h after the induction of colitis. The animals were kept in individual cages, and the score was recorded. The stools were collected and observed, and zero score was given for well-formed pellets, two for pasty and semi-formed stools that did not stick to the anus, and four for liquid stools that did stick to the anus as shown in **Table 1** [10, 11].

TABLE 1: STOOL CONSISTENCY SCORE

S. no.	Indications	Stool consistency score
1	Normal(well-formed pellets)	0
2	Semi formed stool	2
3	Liquid stool	4

After the treatment period, the animals were anesthetized by ketamine hydrochloride (100 mg/kg IP) and the blood was collected from retro-orbital sinus by using a capillary tube into a centrifugation tube which contained EDTA for hematological parameters.

Estimation of Haematological Parameters:

Enumeration of RBC: Using a red blood cell pipette of hemocytometer, well-mixed blood was drawn up to the mark 0.5, and RBC diluting fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 min. The fluid was not allowed to dry. Using high power objective, the red blood cells were counted uniformly in the central squares. The cells were expressed as number of cells $\times 10^6$ / µL [12].

Enumeration of WBC: Well mixed blood was drawn up to 0.5 marks of a white blood cell pipette of hemocytometer and WBC diluting fluid Turk's fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 min. The fluid was not allowed to dry. Using low power objective, the white blood cells were counted uniformly in the larger corner squares. The cells were expressed as the number of cells $\times 10^3$/µL [13].

Estimation of Haemoglobin by Sahli's Acid Haematin Method: 0.1 N HCl was added in the Haemoglobinometer up to the lowest mark by using a pipette. Blood was drawn up to 20 µL in the Sahli's pipette. The blood column was adjusted carefully without bubbles. The excess of blood on the sides of the pipette was wiped off by using a dry piece of cotton. The blood was blown into the acid solution in the graduated tube, and the pipette was rinsed.

Mixed well and allowed the mixture to stand at room temperature of 10 min. The solution was diluted with distilled water by adding a few drops of water carefully and by mixing the reaction mixture until the color matches the color, in the comparator. The lower meniscus of the fluid was noted, and reading was taken in g/dl [14].

Estimation of Packed Cell Volume by Microhematocrit Method: Anticoagulated blood was taken in the Pasteur pipette without any air bubble. The tip of the polythene tube was introduced into Wintrobe's hematocrit tube till it reached the bottom without any air bubble. The blood was filled up to the zero marks. The mouth of the tube was closed with a cotton plug. It was centrifuged for 30 min at a speed of 3000 revolutions/minute. The height of the column of packed cells was directly read from the hematocrit tube and expressed as percentage [15].

Macroscopical Parameters: The animals were sacrificed by cervical dislocation on 11thday, and the colon tissue about 8 cm in length and 2 cm proximal to the anus was excised, opened longitudinally, and washed in phosphate-buffered saline (pH 7.4).

Colon Weight to Length Ratio: Took the weight of the specimens and evaluated the colitis parameters. The intensity of the edema was evaluated by estimating the ratio of wet tissue weight to length of the colon [16].

Ulcer Score: The tissues were mounted on a plain paper, and the inflammation was noted as macroscopic ulcer score using the scale of Morris *et al*., as given in **Table 2** [17].

TABLE 2: MACROSCOPIC ULCER SCORE

S. no.	Indications	Ulcer score
1	No ulcer	0
2	Mucosal erythema	1
3	Mild mucosal edema, slight bleeding or slight erosion	2
4	Moderate edema, bleeding ulcer or erosions	3
5	Severe ulceration, erosion, edema and tissue necrosis	4

Ulcer Index: The ulcer area and total area of the mounted colon were measured [18].

The ulcer index was calculated as follows,

Ulcer index (UI)= Ulcerated area of the colon/total colon area

Ulcer area and total colon area are calculated in mm^2

Percentage of ulcer protection was calculated by

$$\% \text{ of ulcer protection} = Uc - Ut \times 100 / Uc$$

Uc = Ulcer index of the positive control
Ut = Ulcer index of the treated group

Tissues were fixed in 10% formalin saline and examined histopathologically. The remaining tissue was stored at –20 °C for the estimation of biochemical parameters.

Estimation of Biochemical Parameters: The piece of colon reserved for estimation of biochemical parameters was washed thoroughly with ice-cold 0.1 M phosphate-buffered saline (pH 7.4). It was blotted dry and homogenized in 1.15% potassium chloride to prepare a 10% w/v suspension. This suspension was centrifuged at 16,000 × g for 1h in a cooling centrifuge at 0 °C. The supernatant was assessed for superoxide dismutase (SOD) activity, glutathione (GSH) level, and lipid peroxidation (MDA level). The myeloperoxidase (MPO) activity, nitrite, and nitrate level were also determined.

Estimation of Superoxide Dismutase (SOD) Activity: 0.5 ml of supernatant tissue homogenate was taken in a test tube. To this, 1.5 mL of carbonate buffer (pH 10.2), 0.5 mL of 0.1 mM EDTA, and 0.4 mL of epinephrine were added, and the OD was taken at 480 nm. Epinephrine was added just before taking the OD. The activity of SOD was expressed as units/min/mg protein. One unit of the enzyme is defined as the amount of enzyme which inhibits the rate of adrenaline auto-oxidation by 50% [19].

Estimation of glutathione (GSH) Activity: One ml of homogenate was precipitated with 1 mL of TCA, and the precipitate was removed by centrifugation. To 0.5 mL of supernatant, 2 mL of DTNB was added, and the total volume was made up to 3 mL with phosphate buffer. The absorbance was read at 412 nm. The level of glutathione was expressed as µmol/mg protein [20, 21].

Assay of Lipid Peroxidation (LPX): Lipid peroxidation was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS). 0.1 mL of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 mL of TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA in 1:1:1 ratio) and placed in a water bath for 15 minutes and cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm. The level of lipid peroxides was expressed as nmoles of MDA formed/mg protein [22-24].

Myeloperoxidase Assay: Piece of inflamed colon tissue was rinsed with ice-cold saline blotted dry, weighed, and excised. Minced tissue was homogenized in 10 volumes of ice-cold potassium phosphate buffer (pH 7.4) using Remi tissue homogenizer. The tissue homogenate was centrifuged at 3500 rpm for 30 min at 4 °C. The supernatant was discarded. 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) and l0 mM EDTA was then added to the pellet. It was then subjected to one cycle of freezing and thawing and a brief period of (l5s) of sonication. After sonication, the solution was centrifuged at 15,000 rpm for 20 min. Myeloperoxidase activity was measured spectrophotometrically as follows. 0.1 mL of supernatant was combined with 2.9 mL of 50 mM phosphate buffer containing 0.167 mg/mL o-dianisidine hydrochloride and 0.0005% H_2O_2. The change in absorbance was measured spectrophoto-

metrically at 460 nm. One unit of MPO activity is defined as that degrading 1 μmol of peroxide per minute at 25 °C [25, 26].

MPO activity = X / Weight of tissue piece taken

X = 10 × change in absorbance per minute/volume of supernatant taken in the final reaction

Measurement of Nitrite and Nitrate Levels: Total nitrite and nitrate contents both stable end products of NO were measured by spectrophotometric methods with a nitric oxide assay kit. Full-thickness colon samples were pooled, weighed, and homogenized in 10 volumes of phosphate-buffered saline at pH 7.4. The homogenate was centrifuged at 10,000g for 20 min, and the supernatant was filtered. The filtrate was incubated with nitrate reductase for 3 h and assayed for nitrite and nitrate contents with Griess reagent and absorbance was measured at 540 nm [27].

Statistical Analysis: Experimental values were expressed as mean ± S.D, n= 6. Statistical significance (P) calculated by one-way ANOVA followed by Dunnett's t-test as posthoc analysis. P values were calculated by comparing treated group(s) with induced group. In tables, * symbol indicates different level of significance, *** for $P < 0.001$, ** for $P < 0.01$ and * for $P < 0.05$.

RESULTS AND DISCUSSION:

Acute Toxicity Study as per OECD Guidelines 423: There were no mortality and no signs of toxicity. The parameters like body weight, food and water intake remained within the normal range throughout the period of study (14 days), indicating that the extract was safe up to an oral dose of 2000 mg/kg.

Effect of Treatment on Body Weight: The body weights of the animals before and after the treatment are given in **Table 3**.

The results showed a decrease in the bodyweight of Group II animals treated only with acetic acid. In prednisolone treated animals there was a significant increase in the body weight. The body weight was increased for both the doses of extract. The increase in body weight was more significant for SCEL at 500 mg/kg.

TABLE 3: EFFECT OF EXTRACT AND PREDNISOLONE ON BODY WEIGHT

Group	Initial body weight gm	Final body weight gm
Group I	154.792±0.332	165.281±0.938***
Group II	156.619±0.329	149.914±0.78
Group III	152.783±0.184	162.631±0.077***
Group IV	157.937±1.007	159.684±1.118*
Group V	153.733±0.84	160.881±0.293***

The body weight reduction of animals is an indication of their debilitated condition due to colitis. Ulcerative colitis is associated with weight loss due to loss of appetite and diarrhoea [28].

Effect of Treatment on Stool Consistency: The stool consistency score after 24 h of administration of acetic acid in different treatment groups are tabulated in **Table 4**.

TABLE 4: EFFECT OF EXTRACT AND PREDNISOLONE ON STOOL CONSISTENCY SCORE

Group	Stool consistency score
Group I	00.00
Group II	3.67±0.03175**
Group III	0.67±0.02887**
Group IV	2.66±0.03175
Group V	2.33±0.1025

After the administration of acetic acid, an increased score in stool consistency was observed in the positive control group (Group II) of animals when compared with the control group (Group I) of animals. The extract pre-treated groups and the prednisolone drug-treated group showed a decrease in the stool consistency score when compared with the group II animals.

Hematological Parameters: The RBC count, WBC count, hemoglobin level, and packed cell volume of different groups of animals after treatment are tabulated in **Table 5**.

TABLE 5: EFFECT OF EXTRACT AND PREDNISOLONE ON HAEMATOLOGICAL PARAMETERS

Group	RBC X^{10}^6/μL	WBC X^{10}^3/μL	Haemoglobin (g/dL)	Packed Cell Volume (%)
Group I	6.374±0.439	13.504±0.368	16.203±0.546	51.39±2.984
Group II	4.694±0.323**	10.605±0.576***	12.84±0.186***	33.015±4.458***
Group III	6.234±0.16***	13.227±1.136*	15.855±0.56***	49.094±0.775**
Group IV	5.699±0.337*	12.199±0.93^{ns}	13.976±0.401**	44.496±0.271*
Group V	5.857±0.126**	12.528±0.693*	15.135±0.251***	47.021±2.267**

The results of the hematological study demonstrated that the positive control group showed a significant decrease in RBC, WBC, hemoglobin, and platelet volume compared to normal control. Treatment with prednisolone and the extract increased these hematological parameters as compared to the positive control. Ulcerative colitis is associated with exacerbated hematological imbalance.

Macroscopical Evaluation: Photographs of colon and caecum from different treatment groups are shown in **Fig. 2** and **3**.

Group I: Control

Group II: Positive control (Acetic acid)

Group III: Acetic acid + Prednisolone

Group IV: Acetic acid + SCEL 250 mg/kg

Group V: Acetic acid + SCEL 500 mg/kg

FIG. 2: PHOTOGRAPHS OF COLON FROM DIFFERENT TREATMENT GROUPS

Group I: Control

Group II: Positive control (Acetic acid)

Group III: Acetic acid + Prednisolone

Group IV: Acetic acid + SCEL 250 mg/kg

Group V: Acetic acid + SCEL 500 mg/kg

FIG. 3: PHOTOGRAPHS OF CAECUM FROM DIFFERENT TREATMENT GROUPS

Colonoscopic evaluation is an important tool in the evaluation of ulcerative colitis. UC is divided by disease extent into proctitis, proctosigmoiditis, left-sided colitis, and pan-colitis. In addition, a caecal or peri-appendiceal patch and backwash ileitis are associated with UC [29].

Colon Weight to Length Ratio: The colon weight to length ratio is shown in **Table 6**.

TABLE 6: EFFECT OF EXTRACT AND PREDNISOLONE ON COLON WEIGHT TO LENGTH RATIO

Group	Colon weight to length ratio
Group I	127.054±2.257
Group II	295.482±11.589***
Group III	154.68±10.251***
Group IV	226.083±5.014***
Group V	199.655±12.533***

The ratio of colon weight/length was found to be increased significantly in the acetic acid-induced positive control group as compared to the normal group. There was a significant decrease in the colon weight/length ratio in prednisolone-treated groups. The extract, which was given prophylactically, also reduced the colon weight/length ratio significantly.

The wet weight of the inflamed colon tissue is considered as one of the reliable and sensitive indicators of the rigorousness and level of an inflammatory response in acetic acid-induced colitis. The elevation of the colon weight to length ratio in the acetic acid group indicates the damage produced by the AA. Colonic weight/length ratio is an index for local inflammation along with other parameters of edema and wall thickening [9, 10].

Ulcer Score: The effect of prednisolone and prophylactic treatment by the extraction on the ulcer score is shown in **Table 7**.

TABLE 7: EFFECT OF PREDNISOLONE AND EXTRACT ON ULCER SCORE OF COLON

Group	Ulcer score in colon
Group I	0±0
Group II	3.812±0.034***
Group III	1.758±0.115***
Group IV	2.919±0.796^{ns}
Group V	2.207±0.197***

Ulcer score is an indication of mucosal inflammation. The observed ulcer score of acetic acid treated positive control group was found to be high. Prophylactic treatment with the extracts decreased ulcer score of the colon compared to the acetic acid control group.

Ulcer Area of Distal Colon (%): The percentage of distal colon area affected by ulcer is tabulated in **Table 8**.

TABLE 8: EFFECT OF EXTRACTS AND PREDNISOLONE ON ULCER AREA OF DISTAL COLON

Group	Ulcer area of distal colon (%)
Group I	0±0
Group II	7.383±0.516***
Group III	2.422±0.157***
Group IV	4.335±0.502***
Group V	3.192±0.14***

The ulcer area of the acetic acid-treated positive control group was high, indicating the high ulcerogenic effect of acetic acid. There was a significant reduction in the ulcer area of the animals treated with prednisolone. The animals treated prophylactically with the extracts showed a reduction in the ulcer area, revealing the activity of the extracts to protect the colon from inflammatory damage and ulcer formation.

Ulcer Index and Percentage of Ulcer Protection: **Table 9** shows the ulcer index of various groups of animals and the percentage of ulcer protection by prednisolone and the extract in various doses.

TABLE 9: ULCER INDEX AND PERCENTAGE OF ULCER PROTECTION

Group	Ulcer Index	% of ulcer protection
Group I	0±0	00
Group II	16.021±0.627***	00
Group III	6.161±1.34***	62.5
Group IV	9.542±1.461**	41.68
Group V	6.747±1.505***	56.25

The acetic acid-treated positive control group showed the highest ulcer index indicating the high ulcerogenic effect of acetic acid. Prednisolone treatment decreased the ulcer index of colon significantly, and the pre-treatment with the extract also reduced the ulcer index.

Biochemical Parameters: Estimation of Superoxide dismutase (SOD) activity and Glutathione (GSH) level.

The effect of extract and prednisolone on SOD activity and GSH level in the tissue are tabulated in **Table 10**.

TABLE 10: SOD ACTIVITY AND GSH LEVELS IN DIFFERENT GROUPS OF ANIMAL

Group	SOD (Unit/min/mg protein)	GSH (µmoles/min/mg protein)
Group I	0.887±0.013	0.159±0.003
Group II	0.434±0.048***	0.101±0.004***
Group III	0.835±0.034***	0.152±0.004***
Group IV	0.657±0.003**	0.129±0.002***
Group V	0.717±0.03***	0.145±0.004***

The tissue antioxidant enzymes such as SOD and GSH were lowered significantly in the acetic acid administered positive control group compared to normal control animals. The standard drug prednisolone and the extract attenuated the oxidative stress by elevating the SOD activity and GSH level.

Malondialdehyde (MDA) Level and Myeloperoxidase (MPO) Activity: Table 11 shows the effect of extract and prednisolone on MDA level and MPO activity.

TABLE 11: EFFECT OF EXTRACT AND PREDNISOLONE ON MPO ACTIVITY AND MDA LEVEL

Group	MDA (nmol MDA/mg protein)	MPO (µmol/min/mg tissue)
Group I	0.312±0.016	0.231±0.011
Group II	0.834±0.043***	0.503±0.019***
Group III	0.297±0.036***	0.245±0.004***
Group IV	0.427±0.019***	0.325±0.014***
Group V	0.37±0.092***	0.32±0.005**

The pre-treatment with the extract showed protection against lipid peroxidation characterized by a significant decrease in MDA level. Malondialdehyde (MDA) is one of the most abundant aldehydes generated during secondary lipid oxidation, and it is the marker of oxidation. The increased levels of MPO in the colon tissue of acetic acid-treated positive control indicate the neutrophil infiltration and subsequent inflammation. The inhibition of MPO activity substantiates anti-inflammatory role of extracts in preventing UC [30].

Nitrite and Nitrate Level: Table 12 shows the effect of different treatments on the nitrite and nitrate levels.

TABLE 12: NITRITE AND NITRATE LEVEL IN DIFFERENT TREATMENT GROUPS OF ANIMALS

Group	Nitrite (mg/l)	Nitrate (mg/l)
Group I	2.675±0.078	3.421±0.562
Group II	6.323±0.195***	8.726±0.291***
Group III	2.822±0.138***	4.32±0.256***
Group IV	4.716±0.453**	6.108±0.402***
Group V	4.046±0.091***	5.104±0.245***

Nitric oxide is an important mediator in inflammatory and autoimmune-mediated tissue destruction. NO level was assessed by detecting stable metabolites (nitrates/nitrites) in blood using spectrophotometry after Griess reaction. Pre-treatment with the extracts resulted in the reduction of the nitrite /nitrate level compared to positive control.

CONCLUSION: The ameliorative effect on ulcerative colitis of the ethanolic extract of *Syzygium cumini* leaves was evaluated by the *in vivo* method on male albino Wistar rats by acetic acid-induced colitis model. The macroscopical, hematological, and biochemical parameters and the effect of the extract on stool consistency were assessed. The macroscopical features assessed were body weight, ulcer score, ulcer index, colon weight to length ratio, and percentage of colon area affected. In hematological studies, the RBC count, WBC count, hemoglobin level, and packed cell volume were estimated. The biochemical parameters estimated were the enzymatic antioxidant activity by estimation of superoxide dismutase (SOD) activity and non-enzymatic antioxidant activity by estimation of glutathione (GSH) level and by determination of malondialdehyde level. The assessment of inflammation on colon cells was done by myeloperoxidase assay. The nitrite and nitrate levels in the colon cells of colitis induced animals were also estimated. The results obtained from the assessment of various parameters showed that the extract of *Syzygium cumini* leaves ameliorated the ulcerative colitis.

Because of the various adverse effects, complications, and contraindications of current medication, patients have started using complementary and alternative medicines where herbal medicine can contribute much. Many preclinical studies with medicinal plants could prove that those herbs used for treating UC in folk medicine are effective against UC. Hence it is recommended to consume dietary agents with medicinal value to combat diseases like UC.

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REFERENCES:

1. Shalkami AS, Hassan MIA and Bakr AG: Anti-inflammatory, antioxidant and anti-apoptotic activity of diosmin in acetic acid induced ulcerative colitis. Human and Experimental Toxicology 2017; 37: 78-86.
2. Oishi Y and Manabe I: Macrophages in inflammation, repair and regeneration. International Immunology 2018; 30: 511-28.
3. Alves De Almeida AC, De-Faria FM, Dunder RJ, Manzo LPB, Souza-Brito ARM and Luiz-Ferreira A: Recent trends in pharmacological activity of alkaloids in animal colitis: potential use for inflammatory bowel disease. Evidence-Based Complementary and Alternative Medicine 2017; 8528210: 1-24.
4. Saxena A, Kaur K, Hegde S, Kalekhan FM and Fayad R: Dietary agents and phytochemicals in the prevention and treatment of experimental ulcerative colitis. Journal of Traditional and Complementary Medicine 2014; 4: 203-17.
5. Bijauliya RK, Alok S, Sabharwal M and Chanchal DK: *Syzygium cumini* (linn.) - An overview on morphology, cultivation, traditional uses and pharmacology. International Kournal of Pharmaceutical Sciences and Research 2018; 9(9): 3608-20.
6. Pharate K, Shubham B, Holkar K and Katariya T: A review on *Syzygium cumini*. World journal of Pharmacy and Pharmaceutical Sciences 2018; 7: 527-34.
7. Pradhan, M: Phytochemistry, Pharmacology and Novel Delivery Applications of *Syzygium cumini* (L.). International journal of Pharmacy and Pharmaceutical Research 2016; 7: 659-75.
8. Kiesler P, Fuss IJ and Strobber W: Experimental models of inflammatory bowel disease. Cellular and Molecular Gastroenterology and Hepatology 2015; 1: 154-70.
9. Dugani A, Dakhil B and Treesh S: Protective effect of the methanolic extract of *Malva parviflora* L. leaves on acetic acid-induced ulcerative colitis in rats. Saudi Journal of Gastroenterology 2016; 22: 226-33.
10. Mathew MM, Han NV, Murugesan A, Raj EA and Prasanth KG: Evaluation of the protective effect of *Pterocarpus marsupium* on acetic acid-induced ulcerative colitis in rats. Inflammopharmacology 2015; 23:195-01.
11. Thippeswamy BS, Mahendran S, Biradar MI, Raj P, Srivastava K and Badami S: Protective effect of embelin against acetic acid induced ulcerative colitis in rats. European Journal of Pharmacology 2011; 654: 100-05.
12. Sood R: Text book of medical lab technology. Jaypee Brothers Medical Publishers, 1st edition 2007; 204-21.
13. Chung J, Ou X, Kulkarni RP and Yang C: Counting white blood cells from a blood smear using fourier ptychographic microscopy. Plos One 2015; 10: e0133489.
14. Dacie JC and Lewis SM: Practical Haematology. London: Churchill Livingstone, 11th Edition 2011; 5.
15. Jones RF: Determination of packed cell volume by centrifugation. Journal of Clinical Pathology 1961; 14: 198-99.
16. Appleyar, CB and Wallace JL: Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. American Journal of Physiology 1995; 269: 119-25.
17. Zhang W, Pan Y, Qu S, Wang D, Cheng S and Liu X: Anti-Inflammatory effects of an extract of *Polygonum hydropiper* Stalks on 2,4,6-Trinitrobenzenesulphonic acid-induced intestinal inflammation in rats by inhibiting the NF-κB pathway. Mediators of Inflammation 2018; 6029135: 1-10.
18. Minaiyan M, Ghannadi A, Asadi M, Etemad M and Mahzouni P: Anti-inflammatory effect of *Prunus armeniaca* L. (Apricot) extracts ameliorates TNBS-induced ulcerative colitis in rats. Research in Pharmaceutical Sciences 2014; 9: 225-31.
19. Kakkar P, Das B and Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry and Biophysics 1984; 21: 130-32.
20. Ellman GL: Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 1959; 82: 70-77.
21. Moron MS, Depierre JW and Mannervik B: Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochemica et Biophysica Acta 1979; 582: 67-78.
22. Nithya M, Ambikapathy V and Panneerselvam: *In-vivo* antioxidant and enzymatic activity of *Ganoderma lucidum* on mammary cells of DMBA induced sprague dawley rats. International Journal of Current Microbiolgy and Applied Sciences 2015; 4: 69-77.
23. Jiang ZY, Hunt JY and Wolff SP: Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Analytical Biochemistry 1992; 202: 384-89.
24. Zeb A and Ullah F: A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. Journal of Analytical Methods in Chemistry 2016; 9412767: 1-5.
25. Chougule NB, Nitve SA and Koumaravelou K: Phytochemical investigation and screening for inflammatory bowel disease activity of ethanolic extract of kariyat. Pharmacognosy Journal 2018; 10: 602-10.
26. Bradley PP, Priebat DA, Christensen RD and Rothstein G: Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. The Journal of Investigative Dermatology 1982; 78(3): 206-09.
27. Dinc S, Caydere M, Akgul G, Yenidogan E, Hucumenoglu S and Rajesh M: Methylene blue inhibits the inflammatory process of the acetic acid-induced colitis in rat colonic mucosa. International Surgery 2015; 100: 1364-74.
28. Singh KN and Koli PG. Animal models of preclinical drug research on Ulcerative colitis: A review. Journal of the Scientific Society 2018; 45: 80-83
29. Paine ER: Colonoscopic evaluation in ulcerative colitis. Gastroenterology Report 2014; 2: 161-68.
30. Dey YN, Sharma G, Wanjari MM, Kumar D, Lomash V, and Jadhav AD: Beneficial effect of *Amorphophallus paeoniifolius* tuber on experimental ulcerative colitis in rats. Pharmaceutical Biology 2017; 55(1): 53-62.

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