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Research Article

AMELIORATIVE EFFECT OF *Psidium guajava* LEAVES ON ULCERATIVE COLITIS

[1]Dr. Deepa Jose, *[2]Dr. Prasanth B and [3]Dr. Aleykutty N. A

[1]Vice Principal, Nirmala College of Pharmacy, Muvattupuzha
[2]Professor Nirmala College of Pharmacy
[3]Principal, Caritas College, of Pharmacy, Kottayam

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A B S T R A C T

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 leaves of *Psidium guajava,* belonging to the family *Myrtaceae* were evaluated for 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 *Psidium guajava* (PGEL) 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 11th day and the haematological, macroscopical and biochemical parameters were assessed. Pre-treatment with the extract improved the haematological parameters, body weight and the stool consistency score as compared to positive control.

INTRODUCTION

Medicinal and aromatic plants have a significant role in the life of people with great importance in treating diseases. As mentioned in Ashtanga Hridaya all the plants in this earth are considered as medicinal in Indian tradition. In modern drug development, herbal plants and their derivatives have a very important role. Ulcerative colitis is a subtype of Inflammatory bowel disease (IBD), which is a common chronic inflammatory disease of the gastrointestinal tract. It commonly involves the rectum and may extend to involve other parts of the colon (Ordas I *et al*, 2012). 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 (Al-Rejaie *et al*., 2013; Vinay Kumar *et al*., 2013) Ulcerative colitis affects individuals in the second or third decade of life with symptoms of abdominal pain and diarrhoea mixed with blood along with weight loss, fever and anaemia (Danese S and Fiocchi C., 2011). Etiological factors such as genetic, immunological and environmental are associated with the pathophysiology of the disease. Inflammation of the mucosal lining of the colon results from the interaction between different molecular constituents of the cells. 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 (Al-Rejaie *et al*., 2013 ; Saxena *et al*., 2014). The disease is classified by the extent of involvement as distal colitis, extensive colitis and pancolitis (Sninsky, 2010). As the specific pathogenesis underlying inflammatory bowel disease is complex animal models are essential to understand the 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 (Daren low *et al*., 2013).

Psidium guajava L. known as Guava is a medicinal plant belonging to the family *Myrtaceae. Psidium guajava* is a well known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. The leaves and bark of *Psidium guajava* tree have a long history of medicinal uses and is used in this era also. It is a small tree of about 10 meter height with spreading branches that grows on all kinds of soils (Shirur Dakappa Shruthi, 2013 ; Joseph and Mini Priya, 2011). Fruits, leaves, bark and roots of Guava have been used for treating stomach ache and diarrhoea in many countries. Leaves and seeds are used to treat respiratory and gastrointestinal disorders and they are also used as antispasmodic, antiinflammatory, antihypertensive and antidiabetic. The seeds are used as antimicrobial, antiallergic and anticarcinogenic (Barbalho & Machado, 2012 ; Kamath *et al*., 2008).

This objective of the study was to evaluate the efficacy of *Psidium guajava* leaves to ameliorate Ulcerative colitis.

Corresponding author:* **Dr. Deepa Jose
Professor Nirmala College of Pharmacy

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 *Psidium guajava* leaf (Fig: 1). The plant *Psidium guajava* was authenticated by Dr.Sr.Tessy Joseph, HOD, Department of Botany, Nirmala College, Muvattupuzha. Voucher specimen is kept in the herbarium of Nirmala College, Muvattupuzha. The voucher specimen number is NCH/2014/NCP/3812.

The leaves were collected in the month of March 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 *Psidium guajava* (PGEL)

Approval for the animal studies was obtained from the Institutional Animal Ethical Committee (IAEC No: KMCRET/Ph.D/13/2017-18 and CPCSEA No: 685/PO/02/a/CPCSEA).

Acute toxicity study

Acute toxicity study for the ethanolic leaf extract of *Psidium guajava* extract was carried out in accordance with Organization for Economic Cooperation and Development guidelines 423 using female *albino Wistar* rats. The animals received total ethanolic extract of *Psidium guajava* 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 hours. Animals were observed individually during the first 30 minutes with special attention during the first four hours, periodically during the first 24 hours and daily thereafter for 14 days for general behaviour and mortality of the animals. Changes in the body weight and food consumption of the animals were also observed.

Evaluation of ameliorative effect of extracts on Ulcerative colitis

Method of Experiment: Acetic acid induced ulcerative colitis in rats (Elson *et al* .,1995; Patil *et al*., 2012)

Male *albino Wistar* rats with a body weight 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 PGEL respectively and 2 ml of 4% acetic acid solution once intrarectally on 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 hours after the induction of colitis. The animals were kept in individual cages and 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(Mathew *et al*., 2015; Thippeswamy *et al*., 2011).

Table 1 Stool consistency score

Sl. No.	Indications	Stool consistency score
1	Normal(well formed pellets)	0
1	Semi formed stool	2
3	Liquid stool	4

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

Estimation of haematological parameters

Enumeration of RBC: Using a red blood cell pipette of haemocytometer 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 minutes. 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 $x10^6$ / µL **(**Ramnic Sood, 2007).

Enumeration of WBC*:* Well mixed blood was drawn up to 0.5 mark of a white blood cell pipette of haemocytometer 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 minutes. 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 $x10^3$/µL (Sadoun, 2010).

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 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 minutes. The solution was diluted with distilled water by adding few drops of water carefully and by mixing the reaction mixture until the colour matches the colour, in the comparator. The lower meniscus of the fluid was noted and reading was taken in g/dl **(**Dacie J.C.*et al*., 2011).

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 mark. 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 (Jones R F, 1961).

Macroscopical parameters: The animals were sacrificed by cervical dislocation on 11th day 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 (Appleyard & Wallace, 1995).

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 (Morris GP *et al*., 1989).

Table 2: Macroscopic ulcer score

Sl. 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 (Minaiyan *et al*; 2011).

The ulcer index was calculated as follows,

Ulcer index (UI) = $Ulcerated\ area\ of\ the\ colon \div total\ colon\ area$

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

Percentage of ulcer protection was calculated by

% of ulcer protection = $Uc - Ut \div Uc$ x 100

Uc = Ulcer index of the positive control

Ut = Ulcer index of treated group

Tissues were fixed in 10% formalin saline and examined histopathologically. Remaining tissue was stored at –20 ^{0}C for 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 x g for 1h in a cooling centrifuge at 0^0 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: 0.5ml of supernatant tissue homogenate was taken in a test tube. To this 1.5 mL of carbonate buffer (pH 10.2), 0.5 mLof 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% (Kakkar *et al.,* 1984).

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 (Ellman, 1959; Moron, 1979).

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 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 (Nithya *et al*., 2015; Jiang *et al*., 1992; Ohkawa *et al*., 1979).

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 4^0 C. The supernatant was discarded. 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium 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, solution was centrifuged at 15,000 rpm for 20 minutes. 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 spectrophotometrically at 460 nm. One unit of MPO activity is defined as that degrading 1 µmol of peroxide per minute at 25° C (Krawisz *et al*., 1984; Bradley *et al*., 1982*)*

MPO activity = X ÷ weight of tissue piece taken

X= 10 × change in absorbance per minute ÷ volume of supernatent 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 minutes and supernatant was filtered. Filtrate was incubated with nitrate reductase for 3 hours and assayed for nitrite and nitrate contents with Griess reagent and absorbance was measured at 540 nm (Dinc *et al*., 2015).

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 post-hoc 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.

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***
GroupII	156.619±0.329	149.914±0.78
GroupIII	152.783±0.184	162.631±0.077***
GroupIV	151.648±0.143	151.018±0.696***
GroupV	158.414±0.911	159.258±0.923***

The results showed a decrease in the body weight of Group II animals treated only with acetic acid. In prednisolone treated animals there was a significant increase in the body weight. The animals treated with the extract PGEL did not show any significant change in body weight.

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 (Abdallah *et al*., 2008)

Effect of treatment on stool consistency: The stool consistency score after 24 hours 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
GroupII	3.67±0.03175**
GroupIII	0.67±0.02887**
GroupIV	2.00±0.121
GroupV	1.00 ±0.1406*

Administration of acetic acid an increased stool consistency score in positive control group (Group II) of animals when compared with the control group (Group I) of animals. The pre-treatment with extracts and the treatment with prednisolone decreased the stool consistency score when compared with the group II animals.

Haematological parameters: The RBC count, WBC count, haemoglobin level and packed cell volume of different groups of animals after treatment are tabulated in Table 5.

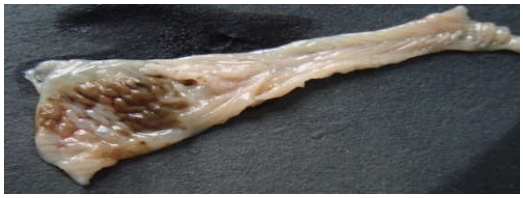
Table 5 Effect of extract and prednisolone on haematological parameters

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
GroupII	4.694±0.323**	10.605±0.576***	12.84±0.186***	33.015±4.458***
GroupIII	6.234±0.16***	13.227±1.136*	15.855±0.56***	49.094±0.775**
GroupIV	5.692±0.073**	11.101±0.242^{ns}	13.477±0.73^{ns}	42.481±2.633*
GroupV	5.83±0.046**	12.064±0.115*	14.452±1.207^{ns}	45.579±2.466**

The positive control group showed, a decrease in RBC count, WBC count, haemoglobin level and platelet volume compared to normal control. These hematological parameters were improved on treatment with prednisolone and the extract when compared to positive control. Ulcerative colitis is associated with exacerbated hematological imbalance.

Macroscopical evaluation: Photographs of colon and caecum from different treatment groups are shown in figures 2 and 3.

Group I Control



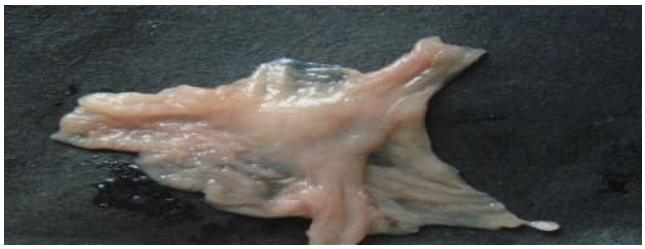
Group II Positive control (Acetic acid)

Group III Acetic acid + Prednisolone

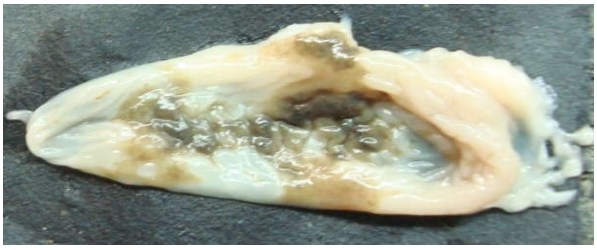
Group IV Acetic acid + PGEL 250 mg/kg

Group V Acetic acid + PGEL 500 mg/kg

Figure 1 Photographs of colon from different treatment groups



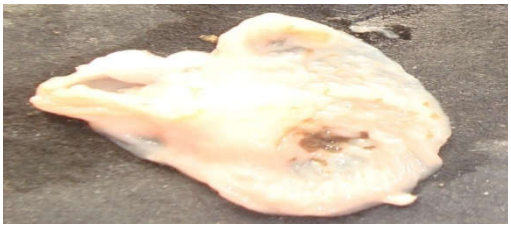
Group I Control



Group II Positive control (Acetic acid)

Group III Acetic acid + Prednisolone

Group IV Acetic acid + PGEL 250 mg/Kg



Group V Acetic acid + PGEL 500 mg/kg

Figure 2 Photographs of caecum from different treatment groups

Colonoscopy is the essential diagnostic tool for suspected Inflammatory Bowel Disease. 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 (Elizabeth R. Paine., 2014; Khawaja F I., 2002)

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
GroupII	295.482±11.589***
GroupIII	154.68±10.251***
GroupIV	262.314±9.463***
GroupV	217.944±6.003***

Increased colon weight/length ratio was shown by acetic acid induced positive control group as compared to normal group. There was a significant decrease in the colon weight/length ratio in prednisolone treated groups. The prophylactic treatment with the extracts 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 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 AA. Colonic weight/length ratio is an index for local inflammation along with other parameters of oedema and wall thickening (Patil *et al*., 2012; Mathew *et al* ., 2015)

Ulcer score: The effect of prednisolone and the prophylactic treatment by the extract 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
GroupII	3.812±0.034***
GroupIII	1.758±0.115***
GroupIV	3.038±0.432^{ns}
GroupV	2.721±0.308**

Ulcer score indicates mucosal inflammation. Positive control group exhibited high ulcer score. Prophylactic treatment with the extracts decreased ulcer score of colon compared to 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
GroupII	7.383±0.516***
GroupIII	2.422±0.157***
GroupIV	5.093±0.379***
GroupV	4.685±0.441***

The increased ulcer area of the acetic acid treated positive control group indicated the high ulcerogenic effect of acetic acid. Prednisolone treatment reduced the ulcer area of the animals significantly. 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
GroupII	16.021±0.627***	00
GroupIII	6.161±1.34***	62.5
GroupIV	10.056±0.199**	37.5
GroupV	7.448±0.329***	52.06

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

Biochemical parameters: Estimation of Super oxide 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
GroupII	0.434±0.048***	0.101±0.004***
GroupIII	0.835±0.034***	0.152±0.004***
GroupIV	0.563 ±0.04**	0.11±0.006
GroupV	0.707±0.012**	0.13±0.003***

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 MDA activity and MPO level

Group	MDA(nmol MDA/mg protein)	MPO(µmol/min/mg tissue)
Group I	0.312±0.016	0.231±0.011
GroupII	0.834±0.043***	0.503±0.019***
GroupIII	0.297±0.036***	0.245±0.004***
GroupIV	0.74±0.171	0.337±0.016
GroupV	0.445±0.21	0.325±0.006

The pre-treatment with the extract showed protection against lipid peroxidation characterised by 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 indicates the neutrophil infiltration and subsequent inflammation. The inhibition of MPO activity substantiate antiinflammatory role of extracts in preventing UC (Dey *et al*., 2017)

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
GroupII	6.323±0.195***	8.726±0.291***
GroupIII	2.822±0.138***	4.32±0.256***
GroupIV	5.197±0.746	8.418±0.548
GroupV	4.838±0.692*	6.345±0.249***

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. Pretreatment with the extracts resulted in the reduction of the nitrite /nitrate level compared to positive control.

CONCLUSION

The ethanolic extract of *Psidium guajava* leaves was evaluated for ameliorative effect on ulcerative colitis of by *in vivo* method on male albino *Wistar* rats by acetic acid induced colitis model. The macroscopical, haematological and biochemical parameters and the effect of the extract on stool consistency were evaluated. The macroscopical features like body weight, ulcer score, ulcer index, colon weight to length ratio and percentage of colon area affected were assessed. In haematological studies the RBC count, WBC count, haemoglobin 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 *Psidium guajava* leaves ameliorated the ulcerative colitis.

The herbal medicines which are used traditionally have been found to be useful in treating many pathologic conditions. Because of the various adverse effects, complications and contra indications of current medication patients have started using complementary and alternative medicines where herbal medicine can contribute much. UC patients require taking medication throughout their lives to prevent relapse, reduce the risk of colorectal cancer and improve quality of life. Many preclinical studies with medicinal plants could prove that those herbs used for treating UC in folk medicine are effective against UC. Even though many herbs are found to be effective in the management of UC, further investigation is needed to prove the long-term efficacies of these herbal medicines.

Conflict of Interest*:* The authors declare no conflicts of interest.

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