

Investigation of *in vitro* and *in vivo* Pursuits of *Laurus nobilis* Extract on Acetic Acid-induced Ulcerative Colitis in Wistar Rats

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ABSTRACT

Background: The ethanolic extract of *Laurus nobilis* possesses an anti-inflammatory effect, which will protect against acetic acid-induced Ulcerative colitis by inhibiting endogenous free radicals.

Objectives: The work has been undertaken to explore the efficacy and safety of ethanolic leaf extract of *Laurus nobilis* in treating acetic acid-induced Ulcerative colitis by estimating the biochemical, physical parameters, and histopathological studies. **Materials and Methods:** The plant powder of *L. nobilis* was extracted using 70% ethanol, and a phytochemical investigation was performed. Male and Female Wistar albino rats were divided into six groups and pretreated with saline, saline followed by ulcer induction, two doses of extracts (100mg/kg and 200mg/kg b.w), and sulfasalazine (one day) followed by ulcer induction and one low dose extract treatment without inducing ulcer. **Results:** The phytochemical investigation of the extract confirms the presence of flavonoids. The biochemical estimations showed increased SOD, CAT, and GSH and decreased levels of TBARS in extract-treated groups compared to the acetic acid-induced group. The physical parameters like Crypt abscesses, gross mucosal inflammation, wet weight of the colon, diarrhea, and activity score have also shown better results in extract-treated groups showing a protective effect. Histopathological results further confirmed the therapeutic effect of the extract. **Conclusion:** The protective effect of ethanolic leaf extract of *Laurus nobilis* has been proved. The therapeutic effect was mainly due to flavonoids, and the low-dose extract was more potent than the high-dose extract.

Keywords: *Laurus nobilis*, Ulcerative Colitis, Anti-oxidant, Histological Studies, Ethanolic Extract.

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INTRODUCTION

Irritable Bowel Syndrome (IBS), which affects 10%-15% of the Western population, and Inflammatory Bowel Disorders (IBDs), whose prevalence is expanding globally.^{1,2} Patients with IBS experience recurring abdominal pain and changes in bowel habits, but there are no visible symptoms of GI inflammation. The most common forms of IBD are Ulcerative Colitis (UC) and Crohn's disease, defined by long-term, chronic inflammation of

the GI tract that causes stomach pain, diarrhoea, intestinal blood loss, and anaemia.³ These symptoms are linked to a significant decrease in quality of life and a significant socio-economic burden due to high healthcare expenditures.⁴ The exact source of the abnormal immune response is unknown. However, dietary and environmental risk factors and host factors, including genetic predisposition and gut microbiota all have a role.^{5,6} Histology is one of many clinical, laboratory, imaging, and endoscopic aspects that are used to make the diagnosis of UC. Erythema, loss of the common vascular pattern, granularity, erosions, friability, bleeding, and ulcerations are just a few of the endoscopic findings. A chronic colonic inflammation also shows these endoscopic characteristics.^{7,8} When making an initial diagnosis, ileocolonoscopy with biopsies is the gold standard.⁹ While treating



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UC, it is important to take into account the endoscopic level of inflammation, the severity of the disease, and prognostic signs for poor outcomes, such as age less than 40 years at diagnosis, the spread of the disease, the existence of extraintestinal symptoms, and response to previous medication.¹⁰⁻¹² In many of these nations, the laurel, *Laurus nobilis* L., an evergreen tree or shrub that is native to southern Europe and the Mediterranean region, is widely cultivated.¹³⁻¹⁵ In the food and beverage industries, the dried leaves and the ethanolic extract made from them are used as spices and flavourings.^{16,17} In Iranian folk medicine, the leaves have been used to treat Parkinsonism, neuralgia, and epilepsy. The fruits and leaves both exhibit aromatic, energizing, and narcotic qualities.¹⁸ Numerous studies have demonstrated the antibacterial and antioxidant properties of laurel ethanolic extract and/or extract. *L. nobilis* leaves have been consumed orally for a long time to treat gastrointestinal symptoms such flatulence and bloating in the epigastrium.¹⁹ Additionally, it has been used to relieve rheumatic pain and haemorrhoids.

Corticosteroids, immunosuppressants, antibiotics, and biologics (Anti-Tumor Necrosis Factor (TNF)- α) are all used in the traditional treatment of UC. However, there are a variety of negative effects associated with using these medications, some of which can be quite serious. Outside of hospitals where conventional medicine is practised, complementary or alternative medicine refers to diagnostic and treatment approaches. UC patients are using more natural products, such as those made from plants and herbal remedies. The purpose of this study was to determine whether an ethanolic leaf extract of *Laurus nobilis* could prevent UC brought on by acetic acid.²⁰

MATERIALS AND METHODS

Chemicals

Sulfasalazine was collected from Natco Pharma Ltd., Hyderabad, and all other chemicals and solvents used were of analytical grade.

Animals

Under this investigation, male and female Wistar rats with a mean body weight of 250 g were housed under conventional settings (27°C, relative humidity 44–56%, and light and dark cycles of 10 and 14 hr, respectively), fed a regular rat diet, and given access to filtered drinking water as needed. The Institutional Animal Ethical Committee (IAEC) of the Annamacharya College of Pharmacy, Rajampet, Kadapa, and the Committee for Control and Supervision of Experiments on Animals (CPCSEA Reg No.1220/a/08/CPCSEA), Ministry of Social Justice and Empowerment, Government of India, gave their approval for all the experiments and protocols detailed in the current study.

Preparation of the extract

In January 2021, *Laurus nobilis* leaves were obtained from the cracks in the rocks in the Tirumala woods in Tirupati, Andhra

Pradesh, India. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, provided additional verification and authentication of the plant sample, and the voucher specimen number (ANCP-MP-COL-04/16) was registered, stored in the herbarium, and kept in our lab for future use. A rotary evaporator was used to remove the solvent after soxhlation to obtain the residue from the powdered dried leaves. For the experiment, doses of 100 and 200 mg of the residue per kg of body weight were determined and dissolved in water.

Antimicrobial activity of Ethanolic extract of *Laurus nobilis* L. leaves

The inhibition halo test was performed to evaluate the potential antimicrobial activity of the *L. nobilis* ethanolic extract.²¹ Selected bacterial strains were obtained from Sri Adichunchanagiri College of Pharmacy's Department of Microbiology. At 37°C for 18 hr, nutrient broth (Sigma Aldrich, Milano, Italy) was utilized as a bacterial growth medium. All cultures were placed onto Nutrient agar plates with their optical densities adjusted to match a 0.5 McFarland standard of 1 10⁸ Colony-Forming Units (CFU)/mL. The ethanolic extract of *L. nobilis* is resuspended in Dimethyl Sulfoxide (DMSO) before being diluted for biological analysis. 0.4, 1, and 2 liters of the ethanolic extract were soaked into sterile filter paper discs (5 mm) and placed on the plates. The diameter of the clear zone visible on the plates (the inhibitory halo zone) was precisely measured prior to incubation at 37°C for 24 hr using a "Extra steel Calliper mod 0289", mm/inch reading scale, precision 0.05 mm, Mario De Maio, Milan, Italy. The negative control was a disc that had only been treated with DMSO. The typical medication was tetracycline (7 g/disc; Sigma Aldrich, Milan, Italy). After three cycles, the experiments were averaged.

Determination of total flavonoid content

The total flavonoid content was verified using the colorimetric method. 1.25 mL of distilled water was added to 0.25 mL of *L. nobilis* leaf extract or (+)-catechin standard solution (20–100 g/mL). After being added, a 75-microliter solution of sodium nitrite (5% w/v) was incubated for 6 min. Before adding 0.5 mL of 1 M NaOH, 150 microliters of a 10% (w/v) aluminium chloride solution were added and incubated for an additional five minutes. The absorbance was determined spectrophotometrically at 510 nm.²²

Antioxidative capacity

Hydroxyl radical scavenging activity

The amount of hydroxyl radicals produced by the Fe³⁺/ascorbate/EDTA/H₂O₂ system was determined by the hydroxyl radical scavenging activity.²³ When the hydroxyl radical reacts with deoxyribose, Thiobarbituric Acid Reactive Compounds (TBARS) are produced. 100 mL of 3.0 mM deoxyribose, 100 mL of 0.1 mM FeCl₃, 100 mL of 0.1 mM EDTA, 100 mL of 0.1 mM ascorbic acid,

100 mL of 1 mM H₂O₂, and 20 mM of phosphate buffer (pH = 7.4) were added to a reaction mixture, and then 1 mL of the mixture was added. An entire hour was spent heating the reaction mixture to 37°C. The test tubes were then filled with 1.0 mL of TCA (2.8%) and 1.0 mL of TBA (1%), and they were then heated for 20 min at 100°C. The mixes' absorption was measured at 532 nm after cooling, and it was compared to a blank that comprised buffer and deoxyribose. The percentage inhibition (I) of deoxyribose degradation was calculated as shown below:²⁴

$$\% I = (A_0 - A_1 / A_0) \times 100$$

Here, A₀ was the absorbance of the control reaction (containing all reagents except the test compound), and A₁ was the absorbance of the test compound.

Inhibition of superoxide radicals

By measuring the formation of Nitro Blue Tetrazolium (NBT), the superoxide radical generation by the xanthine/xanthine oxidase (EC 1.1.3.22) system was evaluated spectrophotometrically.²⁵ A reaction mixture containing 2.0 mL total volume, 100 mL of varied sample concentrations, 100 mL of 2 nM xanthine, 100 mL of 12 nM NBT, 100 mL of 1.0 U/mL xanthine oxidase, and 100 mL of 0.1 M phosphate buffer (pH = 7.4), was added. The absorbance was measured at 560 nm and contrasted to control samples that did not contain the enzyme after 10 min of incubation at 25°C. The percent inhibition of superoxide anion was calculated using the following equation:

$$\text{Inhibition\%} = (A_0 - A_1 / A_0) \times 100$$

In this equation, A₀ was the absorbance of the control, and A₁ was the absorbance of the samples.

Hydrogen peroxide scavenging activity

The ability of the ethanolic extract to scavenge hydrogen peroxide was determined spectrophotometrically as described previously.²⁶ In a buffered phosphate solution of 0.17 M (pH = 7.4), a hydrogen peroxide solution (2 mM) was produced. The three primary components in n-hexane and the ethanolic extract were added in varying proportions to a reaction mixture containing 600 L, 2 mM hydrogen peroxide. The sample was incubated for 10 min at room temperature, and then the absorbance at 230 nm was calculated in contrast to a blank. The following formula was used to determine the % hydrogen peroxide scavenging activity of the samples:

$$\text{Scavenged\% hydrogen peroxide} = (A_0 - A_1 / A_0) \times 100$$

Here, A₀ was the absorbance of the control, and A₁ was the absorbance of the presence of samples.

DPPH assay

After bleaching a purple methanol solution of DPPH, the DPPH test was measured. 100 microliters of the materials (ethanolic extract and three principal components in n-hexane) were combined with five milliliters of a 0.004 percent solution of DPPH in methanol. After 30 min of room temperature incubation, the 517 nm absorbance was measured in comparison to a blank. In order to calculate the DPPH radical scavenging activity, the formula below was used:

$$\text{Inhibition\% of DPPH} = (A_0 - A_1 / A_0) \times 100$$

Here, A₀ was the absorbance of the control, and A₁ was the absorbance of the presence of samples.

Induction of colonic inflammation in rats

Before causing colitis, all animals were starved for an entire night while having unlimited access to water. The colon was reached by inserting a polypropylene tube with a 2 mm diameter through the rectum at a 3 cm distance. Two cc of a 4% (v/v) acetic acid in saline solution was injected. The rats were then kept in a supine Trendelenburg position for 30 sec to stop the intra-colonic instillation from leaking too soon.

Groups

The animals were divided into six groups (n=6 per group).

Group-1: (normal control) received normal saline.

Group-2: (disease control or negative control) animals were induced ulcers using acetic acid.

Group- 3: (standard drug) sulfasalazine (500mg/kg).

Group-4: low dose LNE extract (100 mg/kg).

Group-5: high dose of the extract (200mg/kg).

Group-6: received low-dose extract (100mg/kg) without inducing ulcer.

Exact time of experiment: Pre-treatment has been performed for 30 days (duration of the study)

Route of drug administration: oral.

Oral gavage needles: 16 gauge.

Pre-treatment was performed for Group-3, Group-4 and Group-5 for 30 days. Subsequently, ulcers were induced with acetic acid via the rectal route to Group-2 to Group-5 animals. Group-1 and Group-6 were ulcer-free. The standard sulfasalazine was administered orally to Group-3 by suspending it in distilled water using 1% Carboxy Methyl Cellulose (CMC) since the solubility is 1mg/mL. Low and high dose of extracts were dissolved in sufficient quantity of Millipore water and given orally. After inducing the ulcers, biochemical estimations, histopathology, mucosal inflammation, crypt abscesses, and morphological disease score

Table 1: In vitro antibacterial activity of Ethanolic extract of *Laurus nobilis*.

Sl. No	Microorganisms	Zone of inhibition in mm
1	<i>Aspergillus brasiliensis</i> ATCC 16404	15.1 ± 0.02
2	<i>Bacillus cereus</i> 4313	4.66 ± 0.57
3	<i>Bacillus subtilis</i> ATCC 6633	14.8 ± 0.05
4	<i>Candida albicans</i> ATCC 10231	16.4 ± 0.02
5	<i>Escherichia coli</i> ATCC 8739	12.2 ± 0.04
6	<i>Kocuria rhizophila</i> ATCC 9341	13.6 ± 0.05
7	<i>Pseudomonas aeruginosa</i> ATCC 9027	8.0 ± 0.00
8	<i>Saccharomyces cerevisiae</i> ATCC 2601	33.3 ± 0.00
9	<i>Salmonella abony</i> NCTC 6017	12.2 ± 0.04
10	<i>Staphylococcus aureus</i> ATCC 6538	15.1 ± 0.02

were performed for all the groups. But only two animals from Group-I were sacrificed to study the histopathology and clinical assessment since they are normal controls. In all other groups, all the animals were sacrificed to study the mentioned parameters.

Assessment of colitis

Numerous factors, including diarrhoea, the weight of the colon when wet, the activity score, gross mucosal inflammation, the gross morphological disease score, and crypt abscesses, were used to determine the disease's severity.

Gross mucosal inflammation

By cutting, weighing, and splitting longitudinally 6 cm of colon that extended proximally for 2 cm above the anal orifice, the brain scoring system was used to macroscopically evaluate mucosal inflammation. Each ulcer's macroscopic damage in each rat was graded using a scale that went from 0 to 10 as follows: (0) normal, (1) No ulcer, mild petechiae/ Hypervascularity, (2) No ulcer, moderate petechiae/ Hypervascularity, (3) Ulcer < 1 cm with petechiae/ Hypervascularity, (4) Ulcer < 1 cm with petechiae/ Hypervascularity at two or more sites, (5) Ulcer 2 cm with petechiae/ Hypervascularity, (6) Ulcer 3 cm with petechiae/ Hypervascularity, (7) Ulcer 4 cm with petechiae/ Hypervascularity, (8) Ulcer 5 cm with petechiae/ Hypervascularity, (9) Ulcer 6 cm with petechiae/ Hypervascularity, (10) Ulcer > 6 cm with petechiae/ Hypervascularity.²⁴

Histopathological study

The colons were removed from each group, briefly rinsed with saline solution, and then fixed in 10% buffered formalin. The colons were preserved in 10% buffered formalin before being fixed

in paraffin, sliced into 5 m slices, and stained with hematoxylin and eosin. Then, a light microscope was used to search for the histological changes in these sections. The remaining colons were kept for biochemical estimations.

Biochemical studies

Superoxide Dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH), and Thiobarbituric Acid Reactive Substances (TBARS) were evaluated using the remaining colons. SOD levels in the colons were determined by the McCord and Firdovich method (1969) and modified by Kakkar *et al.*, 1984. Catalase levels were estimated by the method described by Aebi, 1974. Protein estimation for the tissue sample was done by the Bradford, 1976 method. TBARS levels were determined by a modified version of the method described by Ohkawa *et al.*, 1979.²⁷

Statistical analysis

The mean and SEM were used to express all the data. Using a computer-based fitting programme (Prism, GraphPad), the one-way-ANOVA multiple comparison test and Tukey's test were used to determine whether there was statistical significance between more than two groups. The threshold for statistical significance was set at $p < 0.005$.

RESULTS AND DISCUSSION

The antibacterial activity of the ethanolic extract was tested on ten bacterial strains, including Gram-positive and Gram-negative bacteria, with some examples including *Staphylococcus aureus*, *Bacillus cereus* (4313), Gram-positive, and *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-negative bacteria. Table 1 shows the activity's results, specifically the inhibition of diameters estimated in millimeters. The ethanolic extract has substantial antibacterial activity against all microbes even when employed in small amounts (0.4 L), with inhibition zones ranging from 4.66 to 33.3 mm. Given the zone of inhibition generated by tetracycline, such results intrigued us. Two cases of *Escherichia coli*, *Salmonella abony* (12.2 mm), and *Aspergillus brasiliensis*, *Staphylococcus aureus* were inhibited by 0.4 liters of the ethanolic extract (15.1 mm) *Saccharomyces cerevisiae* (33.3 mm) had nearly similar and superior results, followed by *Candida albicans* (16.4% inhibition). Except for *Bacillus cereus*, the ethanolic extract was more efficient against all bacterial strains (4.66 mm). The flavonoid content was calculated as the mean (SD) mg of (+)-catechin equivalents per gram of *L. nobilis* leaf extract. *Laurus nobilis* leaf extract has a total flavonoid concentration of 518.64 (g catechin/ mg sample).

Researchers have found that the ethanolic extract of *Laurus nobilis* L. has antioxidant properties *in vitro*, inhibiting Reactive Oxygen Species (ROS) like the hydroxyl and superoxide radicals, hydrogen peroxide (which is not a free radical but a reactive oxygen type), and 2,2, diphenyl-picrylhydrazyl (DPPH), a stable free radical [Table 2]. Because the findings indicate that the

Table 2: Antioxidant activity of ethanolic extract of *Laurus nobilis*.

Sample	DPPH IC ₅₀ (μL/mL)	Hydroxyl IC ₅₀ (μL/mL)	Hydrogene peroxide IC ₅₀ × 10 ⁴ (μL/mL)	Superoxide IC ₅₀ (μL/mL)
Ethanolic extract of <i>Laurus nobilis</i>	0.576 ± 0.013	0.396 ± 0.028	2.421 ± 0.136	0.141 ± 0.004

Table 3: Gross Mucosal Inflammation (cm), Number of Crypt Abscesses, and Wet weight (g) of isolated Colons.

Treatment Groups	Number of Crypt Abscesses	Gross Mucosal Inflammation (score)	Wet Weight of Colon (g)
Normal control (G1)	0	0	0.64±0.04
Acetic acid (G2)	9.1±1.1***	7.1±0.72###	1±0.1\$\$\$
Sulfasalazine (G3)	1.1±0.63***	2±0.64###	0.8±0.04\$\$\$
LNE 100mg/kg (G4)	3.1±0.72***	2.6±0.41###	0.8±0.1\$\$\$
LNE 200mg/kg (G5)	2.6±1.2***	3###	0.74±0.1\$\$\$
LNE 100mg/kg (G6)	0	0	0.6±0.04

All Values are expressed as mean ± SEM, One Way Analysis of Variance, followed by Tukey's \$\$\$, ###, and *** $p < 0.001$ when comparing Group I and Group VI with control and the remaining groups with disease control.

ethanolic extract derived from laurel leaves may have reactive oxygen species scavenging activity, the Ethanolic extract of *Laurus nobilis* L. and its Main Components Effect on Reactive Oxygen Species Scavenging Activity [Table 3], compares the effects of *L. nobilis* extract and sulfasalazine on crypt abscesses, gross mucosal inflammation, and wet colon weight in comparison to the acetic acid-induced group, as well as the effect of the acetic acid-induced group and a low dose of extract-treated without inducing ulcer to the normal control. Acetic acid generated severe edematous inflammation in the colon, as evidenced by crypt abscesses, a high score of gross mucosal inflammation, and an increase in wet colon weight. LNE (*Laurus nobilis* Ethanolic extract) (100mg/kg and 200mg/kg) and sulfasalazine reduced acetic acid's effect by lowering the number of crypt abscesses, inflammation score, and colon wet weight. These findings were supported by histological findings in Figure 1, which indicated mucosal injury defined by total destruction to the mucosa, goblet cells, submucosa, and muscularis mucosa in acetic acid-treated rats. In sulfasalazine-treated rats, a protective effect against acetic acid-induced colitis was demonstrated, with the outer mucosal layer thickening without injury.

The low dose of the extract showed corrected colon morphology with comparable results to those of a high dose of extract. Outer mucosa was slightly eroded, but inner mucosal regions had not shown any damage signs. In contrast, in high dose extract-treated colon, the damage was observed in mucosal and sub-mucosal areas. Control and low dose extract-treated without inducing ulcer showed a typical colonic environment. Histological studies of colons showed the protective effect of extract against acetic acid-induced colitis [Figure 1]. Acetic acid-induced colon was wholly damaged, eradicated in the sulfasalazine-treated group, and only thickened mucosal layer was observed. Low-dose extract showed comparable results as that of the sulfasalazine with slight

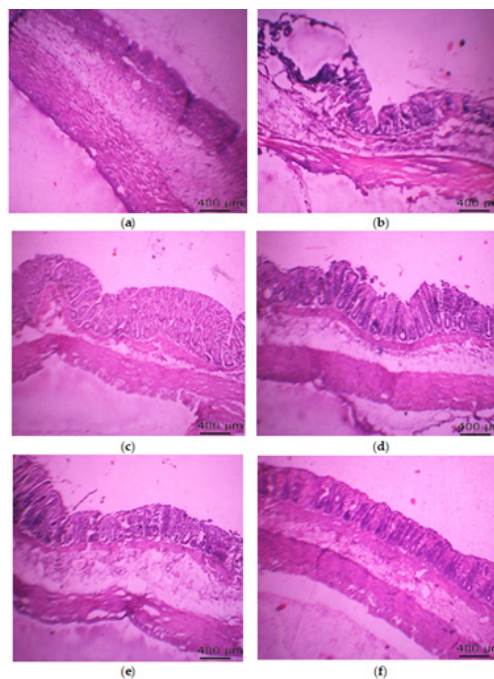


Figure 1: Histologic scoring system. (a) Colonic mucosa of G1, - no possible formation of colonic ulcers – Zero; (b): Colonic mucosa of G2, - there is evidence of the formation of streaks and ulcer spots in streaks – score- 4; (c): Colonic mucosa of G3, there is a complete recovery of ulcer spots or streaks – 1; but 1 or 2 ulcer spots are found in these areas; (d): Colonic mucosa of G4, found notable ulcers and streaks – 1; (e): Colonic mucosa of G5, 2; (f): Colonic mucosa of G6 – zero.

erosions in the outer mucosa. The high-dose extract showed less protective effect than low-dose extract, with damage extending up to the sub-mucosal region.

Biochemical estimations showed that the sulfasalazine and LNE (100mg/kg and 200mg/kg) had decreased the levels of TBARS and increased the levels of GSH, SOD, and CAT compared to that of the acetic acid-induced group [Tables 3 and 4].

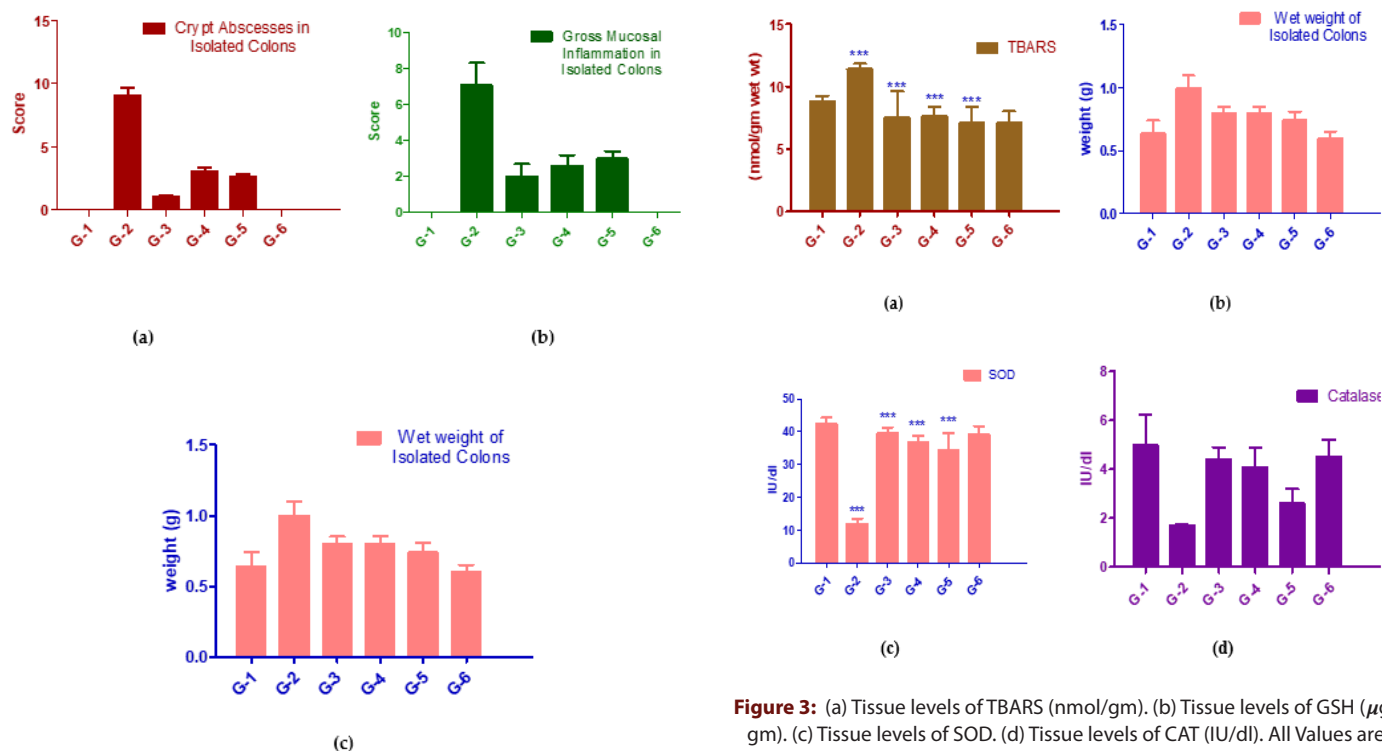


Figure 2: (a) Crypt Abscesses in Isolated Colons. (b) Gross Mucosal Inflammation in Isolated Colons. (c) Wet weight of Isolated Colons.

Figure 3: (a) Tissue levels of TBARS (nmol/gm). (b) Tissue levels of GSH ($\mu\text{g/gm}$). (c) Tissue levels of SOD. (d) Tissue levels of CAT (IU/dl). All Values are expressed as mean $\pm$ SEM, One Way Analysis Of Variance, followed by Tukey's, ** $p < 0.01$ and *** $p < 0.001$ when comparing Group I and Group VI with control and the remaining groups with disease control.

Table 4: Tissue level of TBARS, GSH, SOD, Catalase.

Treatment Groups	TBARS ($\mu\text{g/gm wet wt}$)	TBARS ($\mu\text{g/gm wet wt}$)	SOD (IU/dl)	Catalase (IU/dl)
Normal control (G1)	8.8 $\pm$ 0.43	77.3 $\pm$ 4.9	42.4 $\pm$ 1.9	5.0 $\pm$ 3.9
Acetic acid (G2)	11.4 $\pm$ 0.43**	36.4 $\pm$ 2.3***	11.9 $\pm$ 1.7***	1.7 $\pm$ 0.03
Sulfasalazine(G3)	7.5 $\pm$ 2.1***	73.9 $\pm$ 9.0***	39.5 $\pm$ 1.9***	4.4 $\pm$ 2.5
LNE 100mg/kg (G4)	7.6 $\pm$ 0.78***	69.1 $\pm$ 6.5***	36.9 $\pm$ 1.8***	4.1 $\pm$ 1.8
LNE 200mg/kg (G5)	7.1 $\pm$ 1.3***	73.3 $\pm$ 2.6***	34.5 $\pm$ 5.2***	2.6 $\pm$ 1.6
LNE 100mg/kg (G6)	7.1 $\pm$ 0.92	69.4 $\pm$ 1.3	39.1 $\pm$ 2.6	4.5 $\pm$ 1.3

All Values are expressed as mean $\pm$ SEM, One Way Analysis of Variance, followed by Tukey's test, ** $p < 0.01$ and *** $p < 0.001$ when compared Group II and Group VI with control and the remaining groups with disease control.

Table 5: The animal body weight measurements of all the groups were measured at various time periods.

Test period	Test groups- Average weight in gm (n=6)					
Days	G1	G2	G3	G4	G5	G6
0	251	254	250	252	253	252
15	253	282	263	259	259	254
30	252	279	261	261	255	258

The results proved an improvement in acetic acid-induced colitis in rats treated with the extract compared with the disease-control rats. The number of crypt abscesses, gross mucosal inflammation, and wet colon weight were increased in acetic acid-induced animals compared to that of the extract and sulfasalazine-treated animals [Figure 2]. The body weights of disease control rats were

increased compared with other groups due to significant variation in gross mucosal inflammation [Table 5]. The separated colon tissues of disease-control rats showed a flaccid appearance, wall thickening, and ulcers. The tissues showed a flaccid appearance with hyper-vascularity in standard-treated and extract-treated groups, but ulcers were absent. It was assumed that the decrease

in gross mucosal inflammation in extract-treated rats was due to the inhibitory activity of the extract against COX-2.

The mucosal immune system is mainly affected by cytokines, which majorly produce inflammation.²⁸ It is a well-known fact that TNF- α is abundantly expressed in the gut of IBD patients,²⁹ and the findings proved that mucosal and sub-mucosal inflammation following initial injury was associated with activating arachidonic acid pathways. It leads to the activation of COX-2, which results in more than PGE2. The earlier studies proved that the bay leaf extract possesses COX-2 inhibitory activity.³⁰ In addition, oxidative stress also plays an essential role in the initiation and progression of inflammatory bowel disease. The chronic nature of IBD implies enduring tissue exposure to free radicals. Although the intestinal mucosa contains many endogenous anti-oxidant defense mechanisms, their levels are relatively low compared with those in other organs (e.g., liver, lung). Reactive oxygen species activate nuclear factor kappa-B, which increases TNF- α , producing reactive oxygen species. (Roshni *et al.*, 2010) Thus it was a cyclic process. Inhibition of free radical ions will lead to a decrease in the TNF- α . It has been proved that the ethanolic extract of *Laurus nobilis* has free radical scavenging action.³¹ It had been proved that flavonoids could reduce the free radical formation and scavenge free radicals. Thus, it reduces the TNF- α and facilitates the inflammatory process. Production of Nitric Oxide by iNOS for prolonged periods may also result in the inflammatory response and tissue injury in experimentally induced colitis. Increased NO generation and highly expressed iNOS in the inflammatory regions of the colon may result in the production of macrophages and inflammatory neutrophils as NO production was stimulated by lipo-polysaccharide and IFN- γ .^{32,33} It has been proved that the sesquiterpenes in the *Laurus nobilis* extract possess an inhibitory effect on nitric oxide production in lipopolysaccharide-stimulated macrophages. It is known that intestinal glutathione synthesis was impaired in IBD patients (45). It was observed that GSH levels in the extract-treated and sulfasalazine-treated groups were improved compared with that of the acetic acid-induced group [Figure 3]. With the increase in the levels of GSH and SOD, free radicals may be reduced, and TNF- α levels may be controlled, which results in a decrease in inflammation. The TBARS levels were decreased in extract-treated and sulfasalazine-treated groups compared with the control animals, showing a protective effect against the disease [Figure 3]. The CATALASE levels have not shown any significant variation in extract-treated groups compared with the disease-control group.

CONCLUSION

The study proved that the ethanolic extract of *Laurus nobilis* possesses a protective effect against acetic acid-induced Ulcerative colitis. From *in vitro* antioxidant studies, it was proved that the extract contains antioxidant activity. Phytochemical screening

results showed that the extract has Flavonoids. The biochemical estimations showed increased SOD, CAT and GSH and decreased levels of TBARS in extract treated groups compared to the acetic acid-induced group. All these properties are responsible for the antioxidant activity and the protective effect of the extract against Ulcerative colitis, which is again confirmed by histological studies of colons.

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AUTHOR CONTRIBUTIONS

N.S.: Funding acquisition Methodology. V.C.: Data curation Formal analysis, Writing - original draft. V.S.G.: Investigation Methodology, Writing - original draft. P.G.: Methodology Project administration. N.R.N.: Software Writing - review and editing. S.F.: Methodology. G.M.B.: Writing - review and editing; P.K.: Project administration Resources. M.T.: Writing - original draft. V.C.J.: Validation Visualization.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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