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Research Article

Therapeutic effect of *Nerium oleander* in Acetic acid-induced Ulcerative Colitis model in BALB-C mice

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ABSTRACT

Inflammatory bowel disease (IBD) is a chronic inflammatory intestinal disorder that poses significant challenges in terms of prevention and treatment due to its complex etiology. The multifaceted nature of IBD's underlying causes means that developing effective interventions requires a nuanced understanding and innovative approaches. As a result, research into new drugs and treatment strategies has become a critical area of focus in the scientific community. Among the promising areas of research are phenolic acids, which are abundant in plant-based diets. These compounds have garnered attention because of their potent antioxidant and anti-inflammatory properties, which have shown potential in alleviating colitis. This review aims to provide a comprehensive overview of phenolic acids, highlighting their structures and primary dietary sources, specifically focusing on benzoic acid and cinnamic acid. We delve into the role of phenolic acids as a nutritional therapy for the prevention and treatment of IBD. The review synthesizes findings from animal and cell experiments, which have demonstrated that phenolic acids can effectively mitigate IBD symptoms induced by drug exposure or genetic predispositions. The mechanisms through which phenolic acids exert their beneficial effects include enhancing intestinal mucosal barrier function, reducing oxidative stress, inhibiting the overactivation of the immune response, and regulating the balance of intestinal microbiota. The evidence from these studies underscores the therapeutic potential of *Nerium oleander* in Acetic acid-induced Ulcerative Colitis in BALB-C mice and its amelioration. However, our observations also highlight the necessity for further basic and clinical research to fully understand the capabilities and limitations of phenolic acids and their derivatives as novel therapeutic agents for IBD. Comprehensive investigations will be essential to translate these promising findings into practical treatments for individuals suffering from this debilitating condition..

INTRODUCTION

Inflammatory Bowel Disease (IBD) is a chronic condition of the intestinal tract characterized by

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abnormal immune responses to gut microflora. It comprises two types: Ulcerative Colitis (UC), and Crohn's Disease (CD), which results in transmural ulceration of any part of the gastrointestinal tract. Both UC and CD have varying extents and locations of involvement [McDowell et al., 2024]. CD affects the mouth, anus, and entire layers of the intestine, while UC primarily targets the mucosal layer of the colon [Qin, 2023]. Both conditions present similar symptoms ranging from mild to severe, potentially life-threatening. UC often manifests with blood in stool, severe pain, and diarrhoea, whereas CD also involve bleeding in severe cases, though rectal bleeding is less common. Over 50% of CD patients experience folate and vitamin D deficiencies, while the same percentage of UC patients suffer from iron deficiency [Seyedian et al., 2019].

UC, as first described by Samuel Wilks in 1859, is a chronic multifaceted inflammatory condition with a substantial impact on the quality of life (QOL) of the affected persons [Bellone et al., 2023]. It typically involves periods of flare-ups, where symptoms worsen, followed by periods of remission, where symptoms improve or disappear. These fluctuations can disrupt daily activities, work, and social life, leading to uncertainty and anxiety about when the next flare-up might occur [Agrawal et al., 2022 and Seidelin et al., 2013].

Several genetic variations have been identified that increase susceptibility to UC. These genetic factors influence the immune response, barrier function of the intestinal epithelium, and interactions with gut microbiota [Mukherjee et al., 2018].

UC can be induced by deletion, mutation. Environmental factors including: smoking. Smoking affects ulcerative colitis differently: it worsens the condition for most, but former smokers and some smokers may experience a positive impact. Various factors, such as immune responses, gut function, and inflammation,

contribute to this complex relationship [Birrenbach and Böcker, 2004]. UC mostly affects non-smokers and former-smokers. The effect of smoking shows some differences between male and female patients. Only about 10–15% of UC patients are current smokers, significantly lower than the 25–40% in a control group [Bastida and Beltrán, 2011].

A meta-analysis found that current smokers have a much lower risk of UC compared to lifetime non-smokers, with an odds ratio of 0.41. So, in contrast, UC patients who quit smoking may face higher relapse risk [Cosnes, 2004]. Diet are also the keys in UC development, improved living conditions have led to higher consumption of fatty, protein-rich, and dairy products, along with decreased dietary fibre intake [Mentella et al., 2020]. Particularly, increased trans-unsaturated fat intake heightens UC risk. [Guo and Wang et.al., 2023].

The composition and function of the gut microbiota evolve over a person's lifespan [Rodríguez et al., 2015]. The gut microbiota, being the largest component of the human microbiome, forms a diverse, complex, and stable ecosystem crucial for human health. Its interactions are vital for maintaining the integrity of the intestinal mucosal barrier [Guo et al., 2020]. Age-related changes in the gut microbiome may influence susceptibility to UC and contribute to the different age peaks observed [Badal et al., 2020].

Research on intestinal microbiota shows a significant reduction in Bacteroidetes and Firmicutes, resulting in decreased biodiversity and dysbiosis in affected individuals [Porter et al., 2024]. The overuse of antibiotics can disrupt gut flora and trigger immune disorders and ultimately induced UC [Yoon and Yoon, 2018], it seems that disruptions in the balance between gut microbiome, epithelial barrier, and immune response lead to mucosal damage [Schou et al., 2020]. Mucus acts as a protective barrier between



the microbiota and the epithelium [Cornick et al., 2015].

Genome wide association studies (GWAS) have pinpointed genes that associated with UC such as NOD2, involved in bacterial immune responses and IL23R linked to inflammation [Kurilshikov et al., 2021], which regulate responses to changes in the gut microbiome. Dysbiosis, characterized by altered microbial composition, is observed in UC patients [Alshehri et al., 2021]. Toll-like receptors-2/TLRs 2 and 4 exhibit increased expression in active UC, though their causal relationship with inflammation is unclear. UC is associated with Th2 polarization, evidenced by interleukins-5/IL-5, IL-4, and IL-13 expression, with IL-13 potentially mediating epithelial cytotoxicity, with increased cytokine expression observed in active UC [Ungaro et al., 2017].

In active UC patients, there is a notable imbalance in Th1/Th2 cells, leading to ongoing activation of inflammatory cells within the colon lining. This results in heightened levels of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF- γ , with TNF- γ being particularly prominent. Additionally, there's an increase in the IL-1/IL-1 receptor ratio within colonic tissues, further indicating the inflammatory state characteristic of UC [Strober and Fuss, 2011].

UC is more prevalent globally than CD [Gajendran et al., 2019]. Although incidence is rising in Asia, ranges from 1.2 to 20.3 cases per 100,000 people annually, with a prevalence of 7.6 to 245 cases per 100,000 per year [Feuerstein and Cheifetz, 2014]. In some regions or populations, the incidence might be higher, reaching up to 20.3 cases per 100,000 person/years [Zhao et al., 2019]. Ashkenazi Jews have a reported the incidence of 2-4 times higher than the general population, although some North American population studies contradict this claim [Roth et al., 1989].

UC patients have a certain genetic susceptibility, about 12% of UC patients have a family history

[Santos et al., 2017], twin studies reveal a stark difference, with monozygotic twins showing 6-18% for UC cases [Ryan et al., 2014], with the incidence rate of Caucasians being 3 times higher than that of blacks [Richardus; Kunst, 2001], and predominantly patients of Western and Northern Europeans [Kingwell et al., 2013]. These rates can also change over time due to factors such as changes in lifestyle, diet, or environmental conditions [Gajendran et al., 2019], typically affects individual in two age peaks: the second and third decades and between 50-80 years old [Segal et al., 2021]. Traditionally, IBD exhibits a bimodal incidence pattern, with a primary peak in the 15–25 years of the age group and a secondary, smaller peak in the fifth to seventh decades [Karlinger et al., 2000].

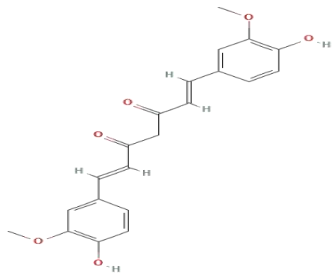
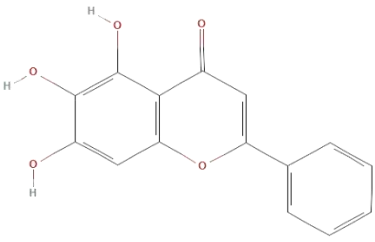
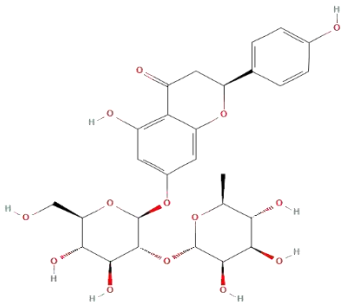
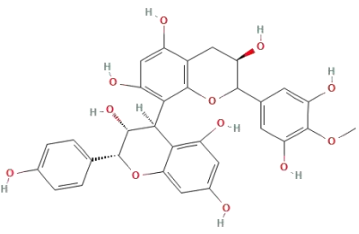
However, there is growing evidence suggesting that the age-specific pattern for both diseases might be changing, especially for UC in men [Rustgi et al., 2020]. Whether this apparent change reflects a real shift in the pattern or is due to the improved robustness of later studies remains uncertain. The recent incidence pattern for UC differs somewhat, with the peak incidence still observed in the 20–29 year age group [Johnston; Logan et al., 2009].

Review of the Literature

Current treatments for IBD can have many side effects, such as fatigue, nausea, abdominal pain, and diarrhoea, vomiting [Rogler, 2010]. Interestingly, dietary supplements of plant-derived natural compounds are considered to have therapeutic protective and therapeutic effects for UC [Farzaei, 2016]. There are many plants phenolic compounds such as Curcumin, Baicalein, Naringin, Proanthocyanidin etc. [Shahidi and Yeo, 2018; Dai et al., 2022] have shown their anti-inflammatory effect and effective treatment in acetic acid-induced UC in mice (Table1) [Saxena et al., 2014].



Table 1: List of plant derived phenolic compounds in the treatment of DSS-induced colitis in mice.

Phenolic Compound	Plant name	Mechanism of action	Reference
 Curcumin	<i>Curcuma longa</i>	Reduces the severity of DSS-induced colitis by inhibit the DNA binding of STAT3 and suppress STAT3 phosphorylation in the mouse colon	Yang et al., 2013
 Baicalein	<i>Berberis species</i>	It ameliorated clinical severity in DSS-induced chronic relapsing mice by inhibiting the differentiation of Th17 cells by downregulating the phosphorylation of STAT3	Yao et al., 2020
 Naringin	<i>Citrus family</i>	It significantly activated DSS-induced PPAR gamma and subsequently suppressed NF-κB activation. The activation of MAPK and NOD like receptor family pyrin domain containing 3(NLRP3) inflammasome by naringin	Cao et al., 2018
 Proanthocyanidin	<i>Malus pumila</i>	Significantly attenuated the expression of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β, and elevated the expression of anti-inflammatory cytokine IL-10 in the colon tissues and serum of DSS-induced colitis mice by suppressing NF-κB signaling pathway.	Chu et al., 2024

According to Dey and Chaudhuri, 2015, *Nerium indicum* is a medicinal plant, used in the treatment of diverse ailments including various chronic inflammatory diseases in traditional medicine. It has been reported that plants containing a high number of flavonoids and phenolic compounds that showed good anti-inflammatory potentials while ethanolic extract of *N. oleander* also

possessed a rich quantity of such phytochemical constituents responsible for these activities. Therefore, this study aimed to confirm the anti-inflammatory/ anti-colitis activity of *N. oleanders* leaf in DSS-induced mice model.

Pathophysiology of UC

The immune system's role in UC development is crucial, as disruptions in gut immune regulation or abnormal responses are key factors in UC

pathogenesis. This can result from unhealthy interactions between gut microflora and mucosal immunity in genetically susceptible individuals [Peterson et al., 2015]. Mucosal immune system

components, including epithelial cells, innate lymphoid cells, and cells of innate/adaptive immunity, along

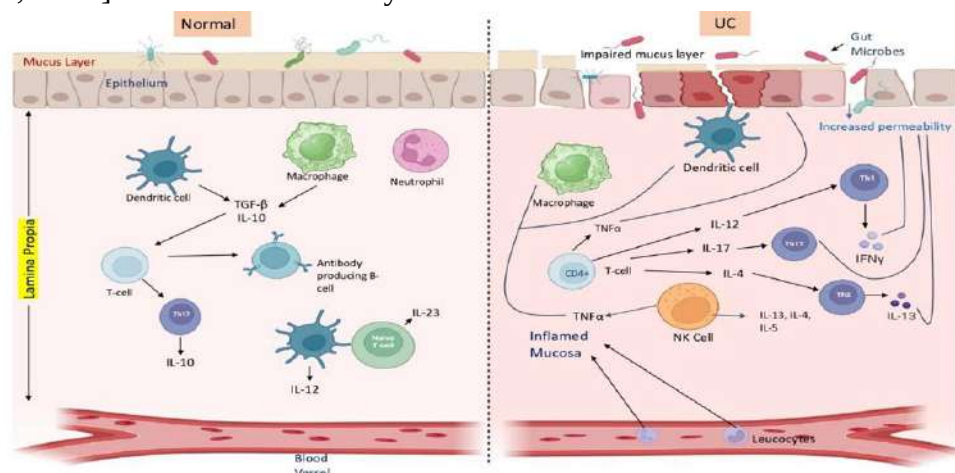


Figure 1: Mucosal immune system components, including epithelial cells, innate lymphoid cells, and cells of innate/adaptive immunity, along with cytokines/chemokines, contribute to IBD pathogenesis. In the left side with normal intestinal condition & in the right side with abnormal immune response.

with cytokines/chemokines, contribute to IBD pathogenesis. Mucosal susceptibility or impaired gut antigen sampling, possibly via autophagy, triggers innate immune response activation, potentially via toll-like receptors (TLRs). Antigen-presenting cells (APC) drive naïve T-cell differentiation into T-helper/Th1, Th2, and Th17 cells that disrupts gut homeostasis and promoting IBD while when external antigens invade the organism [Wallace et al., 2014]. Active UC patients exhibit Th1/Th2 cell imbalance, with constant activation of inflammatory cells infiltrating the colon lining. Elevated levels of IL-1, IL-6, IL-8, and TNF- γ , particularly TNF- γ , alongside an upsurge in IL-1/IL-1 receptor ratio, characterize colonic tissues (Figure 1). Initially dominated by IL-4, the inflammatory response in UC shifts to IL-13 with prolonged inflammation, leading to epithelial ulcer-associated enema [Pelaseyed et al., 2014]. Innate immunity's crucial defence is led by innate lymphoid cells (ILCs), exhibiting diverse cytokine profiles akin to CD4+ T-helper cells. Enriched in mucosal surfaces, ILCs' behavior is shaped by the microenvironment

and cytokine milieu, vital for their function. ILCs must balance responses to environmental antigens with rapid reactions to pathogens, prompting intense research into their plasticity mechanisms. So, in contrast, Innate lymphoid-like cells (ILCs), crucial for innate immunity, reside at the intestinal mucosa surface, maintaining homeostasis and combating inflammation [De Salvo et al., 2020].

Mechanism of pathogenesis

NF- κ B pathway

The inflammatory response involves activating signalling pathways to regulate both pro- and anti-inflammatory factors in tissue cells and recruited leukocytes. Research primarily focuses on IL-1, TNF (Tumour necrosis factor) receptors, and Toll-like receptors (TLRs), which detect microbial patterns. Interleukins-1/ IL-1 and TNF α are key proinflammatory cytokines released during injury or infection. TLRs constitute a recognition system triggering inflammation in response to pathogens and initiating downstream signalling [Lawrence, 2009]. Intestinal inflammation is increased in mouse models of ulcerative colitis. NF- κ B P-p65 abundant in colonic tissues. Loss of regulatory

factors on NF- κ B pathway leads to mucosal changes. Inflammation worsened by inflammatory factor stimulation, activating NF- κ B. NF- κ B expression in colonic tissues correlates with UC severity.

Imbalance in gut bacteria, like Bacteroides, boosts harmful Lipopolysaccharide/LPS. This toxin leaks into circulation, activating TLR4, sparking inflammation via cytokines like TNF- α . Ultimately, it damages the gut barrier. HIF-1 α (Hypoxia inducible factor-1 α), COX-2 (Cyclooxygenase-2), IL-6, IL-1 β , and TNF- α are influenced by NF- κ B and induce and regulate the development of body immunity and inflammation through different pathways [Xiong et al., 2022]. NF- κ B influences HIF-1 α , COX-2, IL-6, IL-1 β , and TNF- α , shaping body immunity and inflammation via diverse pathways. In hypoxic conditions, HIF-1 α surges, guiding neovascularization and fuelling responses like inflammation. In intestinal inflammation, HIF-1 α thrives due to local hypoxia, boosting IL-10 and COX-2 synthesis, thus intensifying inflammation. COX-2, pivotal for prostaglandin synthesis, is notably abundant in UC mucosa, potentially elevating prostaglandin E2 levels [Liu, 2015 and Sheu, 2015]. Cyclooxygenase-2 spikes during cell activation or viral infections, sparking inflammation and tweaking the immune response. TNF- α initiates UC pathogenesis, sparking inflammation and triggering C-reactive protein release, exacerbating the response. It also disrupts vascular microcirculation by inducing intestinal epithelial cell apoptosis, hindering ulcer repair. IL-6, alongside TNF- α , fuels the inflammatory cascade in UC, while IL-10 acts as a potent anti-inflammatory agent, dampening UC-associated inflammation [Xuan-Qing and Chen et al., 2020].

TLR4 in UC

TLRs detect PAMPs (Pathogen associated molecular pattern) like CpG, lipoproteins, LPS, and flagellin, activating immune cells to release

cytokines and trigger an adaptive immune response against pathogens [Dai et al., 2022]. TLR signalling mimics IL-1R pathways, needing TIRAP, protein kinase, and a transcription factor to relay signals. Except TLR3, MyD88 triggers NF- κ B and MAPK for inflammation.

TIRAP links TLR1, 2, 4, and 6 to MyD88, while TRIF joins TLR3 and TLR4. TLR signalling splits into MyD88-dependent and TRIF-dependent pathways (Figure 2). NF- κ B and IRF regulate target genes, producing cytokines and IFNs, enhancing pathogen clearance and inflammation [Lim and Staudt, 2013]. TLR4, widely expressed in various immune cells and epithelial/endothelial cells, is a key member of TLR family and a classical mediator of inflammation and acts as a signalling molecule between innate and adaptive immunity, as well as between inflammation and infection [Akira, 2011; Li et al., 2014]. TLR4 combined with LPS binding protein (LBP), CD14 and MD-2, acts as PRR for LPS of gram-negative bacteria [Kordjazy et al., 2018]. When LPS is identified, LBP will transfer LPS to cell surface CD14 and then bind to the TLR4/MD-2 receptor complex [Pandey et al., 2018]. Subsequently, TLR4 is activated by the formed LPS–LBP–CD14 complex, signalling through adaptor protein myeloid differentiation 88 (MyD88) and serine kinase IL-1R-associated kinase 4 (IRAK4) and the adaptor protein TNF receptor-associated factor 6 (TRAF6). Then, NF- κ B and mitogen-activated protein kinase (MAPK) are activated by this pathway, leading to the transcription of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and IL-6, -8, and -12. These findings have significant implications for the development and severity of inflammatory bowel diseases (IBD).

Under normal conditions, normal or low expression of TLR4 in intestine controls inflammation, but elevated TLR4 expression in the intestinal mucosa of patients with IBD leads to



sustained secretion of inflammatory cytokines and ultimately to the development of intestinal inflammation [Duchmann et al., 1995; Lange et al., 1996]. However, the occurrence and development of IBD may be related to the

abnormal expression of TLR4 in intestinal epithelium. Therefore, targeting and inhibiting TLR4 is effective for the treatment of IBD.

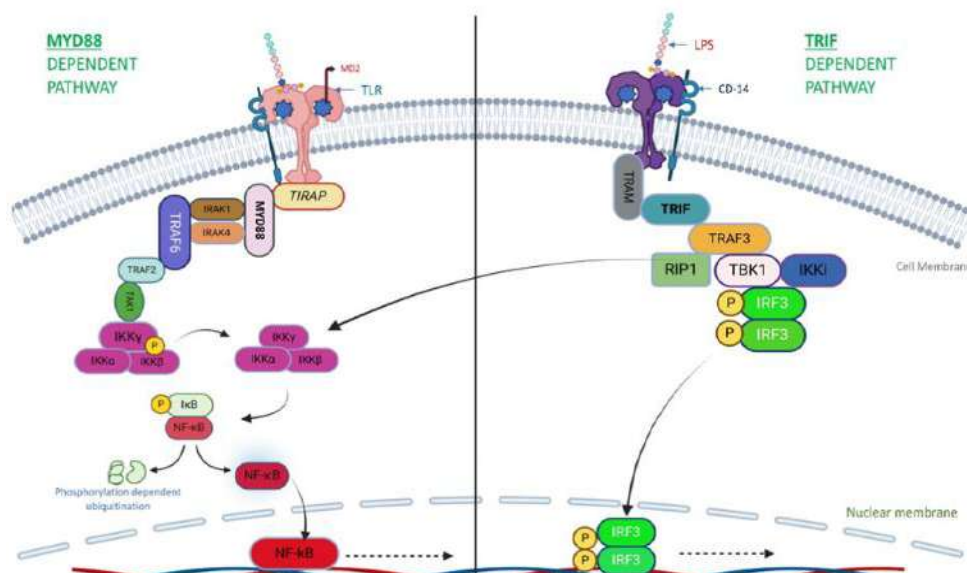


Figure 2: TLR4 detects PAMPs like LPS activating immune cells to release cytokines and trigger an adaptive immune response against pathogens.

MATERIALS AND METHOD

The following protocol outlines how to induce colitis in mice using acetic acid. Due to the essential role of the microbiota in this colitis model. When investigating colitis susceptibility in wild-type, genetically deficient, and/or transgenic mice, it is strongly recommended to use littermates [Chassaing et al., 2014]. Acetic acid-induced disease develops faster in young mice. This may be due to younger mice having lower food and water intake, which delays the onset of gut inflammation. The best age for inducing acetic acid colitis is 6-8 weeks. Both male and female mice develop acetic acid colitis effectively, but males tend to develop more severe and aggressive disease faster than females.

Induction of UC

To develop an animal model of UC, BABL-C mice will be utilized following the established laboratory procedures described by Millar et al. in 1996. UC will be experimentally induced by

administering acetic acid intrarectally. A 1% to 2% acetic acid solution with a pH of 2.3 will be used. This solution will be delivered using a 5FG feeding tube, which is lubricated with a sterile lubricant for easy insertion. The tube will be gently inserted 5 cm into the anorectal tract of the rat. After administering the acetic acid solution, 2 ml of air will be injected to ensure the solution spreads thoroughly throughout the colon. The mice will be positioned upright during this process to aid in the distribution of the solution. Immediately following the injection, the mice will be placed in the Trendelenburg position, which involves laying the animal on its back with its pelvis higher than its head, to further facilitate the spread of the acetic acid.

Disease activity index (DAI)

The disease activity index (DAI) was determined by adding up the scores for weight loss, diarrhoea, and bleeding, following the guidelines established by Chassaing et al., 2014. The weight loss scores

were determined as follows: 0 for no loss; 1 for 1–5% weight loss; 2 for 5–10% weight loss; 3 for 10–15% weight loss; and 4 for more than 15% weight loss. The appearance of blood in the stool was measured by a score from 0 to 4, defined as follows: 0 for no blood; 2 for positive hemocult; and 4 for gross bleeding. The severity of diarrhoea was given a score from 0 to 4, defined as follows: 0 for well-formed pellets; 2 for pasty and semi-formed stools; and 4 for liquid stools.

Weight Loss –

Days	Observation
0	27gm
1	25gm
2	21gm

Appearance of blood in stool –

Days	Observation
0	0
1	3
2	4

Severity of diarrhoea –

Days	Observation
0	0
1	2
2	4

Experimental design for optimization of disease condition

The RSM based optimization involves a six-factor Box-Behnken Design consisting of 54 different

runs. Six different parameters that are used as factors including the variable range were initial body weight (BW, ranges 23–30 gm), acetic acid concentration (AC, ranges between 1 and 2%), acetic acid volume (AV, 1–2 ml), number of doses (D, 1–3 dose), period of induction (P, 6–42 h), and time of holding in Trendelenburg position (T, 1–2 min) (Table2). Each variable had two levels i.e., lower and upper limits as described in. The dependent response selected against these variables is weight loss (WL), the severity of diarrhea (DS), the appearance of blood in the stool (BS), and finally ulcerative colitis (UC). To carry out the experimental runs, $n = 4$ BABL-C mice are used for induction of UC as per the designed experimental parameters. Upon obtaining the experimental results from each run, a separate table was assigned for optimization of the UC condition. In this design, the previously established runs were included followed by only one dependent response i.e., the severity of ulcerative colitis (UC). The experimental value of the severity of ulcerative colitis (UC) was determined based on the average value of weight loss (WL), the severity of diarrhea (DS), and the appearance of blood in the stool (BS) from the previous experiment. Multiple linear regression analysis was studied and experimental data were fitted to a second-order polynomial model.

Table 2: List of various factors and their value limits

Factor	Name of the factor	Abbreviation	Unit	Minimum	Maximum
A	Initial body weight	BW	gm	25	30
B	Acetic acid concentration	AC	%	1	2
C	Acetic acid volume/mice	AV	ml	.5	2
D	Period of induction	P	hour	6	42
E	Time of holding in Trendelenburg position	T	minutes	1	5

Six variable independent factors

- Body weight (BW, ranges 23-30gm).
- Acetic acid concentration (AC, ranges 1-2%).
- Acetic acid volume (AV, ranges 1-3ml).
- Period of induction (P, ranges 6-42h).
- Time of holding in Trendelenburg position (T, ranges 1-5 min).

Three Observations

- Weight Loss.
- Appearance of blood in stool.
- Severity of diarrhoea.

Optimized parameters

Body weight

27gm

Acetic acid
concentration

2%

Acetic acid
volume

0.5ml

Period of
induction

24h

Time of holding in
Trendelenburg
position

1min



1. Insertion of 5FG lubricated feeding tube (5cm) inside the anorectal cavity



Assessment of Ulcerative colitis model

A.

Disease Activity Index

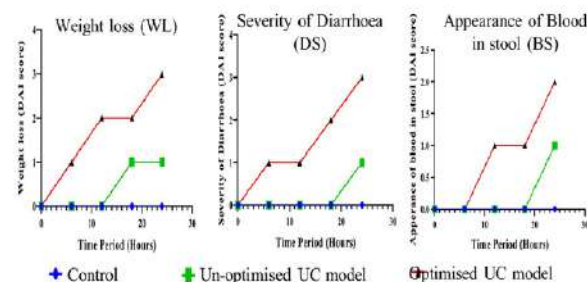


Figure 3: Schematic representation of the work-flow of the methodology used for the optimization of acetic acid-induce UC in BABL-C mice and its DAI.



Doses Optimization of Nerium oleander

A white flower variety of *N. oleander* was collected in February from the University of Burdwan (23.2393° N, 87.8512° E), India, and identified by Prof. Rajib Bandhupadhyay. A voucher specimen is stored in the university's herbarium. The leaves were washed, dried at room temperature for 2 weeks, and ground into powder. The powder (100 g) was mixed with 70% methanol (1,000 ml) and incubated overnight (12 h, 37°C, 160 rpm). After centrifugation at 5,000 rpm for 15 min, the process was repeated. The combined supernatant was filtered, concentrated using a rotary evaporator under reduced pressure, lyophilized, and stored at -20°C. The extract was found to have high phenolic and flavonoid content and strong free radical scavenging capacity.

According to Madaan et al., 2011, 10 mg of gallic acid was dissolved in 100 ml of 50% methanol (100 µg/ml) and further diluted to 6.25, 12.5, 25, or 50 µg/ml. 1 ml of each dilution was mixed with 10 ml distilled water, 1.5 ml Folin-Ciocalteu's reagent, and 4 ml of 20% Na₂CO₃. The volume was adjusted to 25 ml with distilled water, agitated, and left for 30 minutes at room temperature. Absorbance was measured at 720 nm using a UV/VIS spectrophotometer against distilled water. 1 gm of methanol extract was added to 15 ml of 50% methanol, extracted three times by maceration for 2 hours, filtered, and the volume made up to 100 ml with 50% methanol. One ml of the sample was treated similarly to the standard and absorbance measured at 720 nm. The

same procedure was used for the ethyl acetate fraction of the methanol extract. Quantification was based on a standard curve of gallic acid.

The total phenolic content of the extracts was determined according to Hassan et al. A volume of 0.5 mL of extract aliquot was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent and allowed to stand for 5 minutes. Following that, 2 mL of 7.5% sodium carbonate was added. The reaction was kept for 30 minutes, after that the absorbance of each sample was measured at 720 nm using PerkinElemer Lambda 25 UV/VIS spectrometer. A triplicate set of tests were conducted for each sample extract. The total amount of phenolic compounds of each extract was reported as gallic acid equivalents (GAE) milligram per gm.

Table 3: The Concentrations, and OD values for standard gallic acid curve

Concentration(µg/ml)	Absorbance (OD at 720nm)
10	0.05
20	0.07
30	0.12
40	0.2
50	0.26
60	0.29
70	0.37
80	0.38
90	0.39
100	0.47
110	0.54
120	0.55
130	0.7
140	0.6
150	0.74
160	0.77
170	0.79
180	0.8
190	0.79
200	0.86

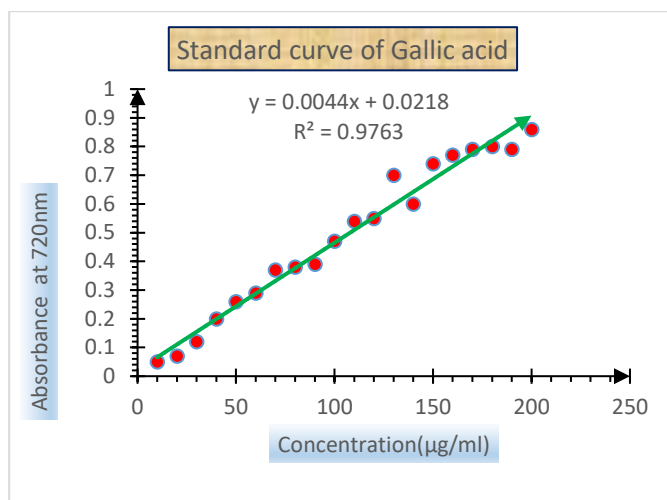
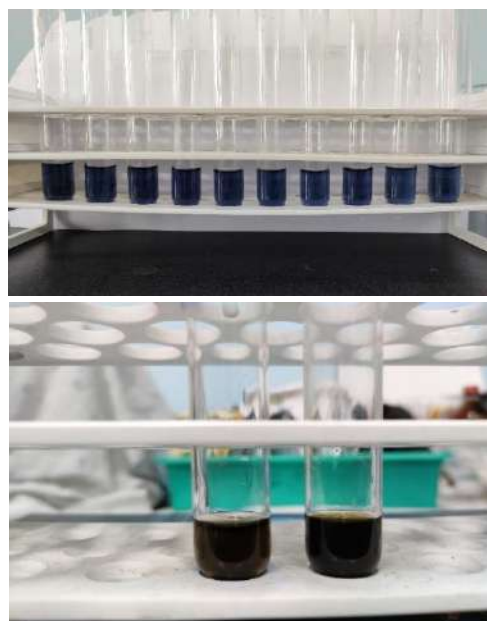


Figure 4: A) Standard Gallic acid curve showing OD at their different concentration.

Table 4: Calculation Of the phenolic compound concentration of an unknown sample.

Unknown Samples	(OD at 720nm)	Concentration (µg/ml)
Sample1 (50µl+50µl)	0.86	190.5



Dose feeding and result

After dose preparation, mice are feed with extract (190µg/ml) by oral gavage feeding tube and keep it for 4 days.



Figure 5: Oral gavage feeding tube



Figure 6: Left-side shows Oral gavage feeding process and right-side shows Recover from UC after 3 days of feeding as result

CONCLUSION

Inflammatory Bowel Disease (IBD) is a chronic intestinal condition that can lead to colorectal cancer if untreated. It includes Ulcerative Colitis

(UC) and Crohn's Disease (CD), causing ulcers in the gastrointestinal tract. Current IBD treatments often have side effects like fatigue, nausea, abdominal pain, and diarrhea. Plant-derived

compounds, such as Curcumin, Baicalein, Naringin, and Proanthocyanidin, show promise in treating UC, particularly in mouse models using acetic acid to induce colitis. This model is popular due to its speed, simplicity, and reproducibility. UC is induced in mice using a 1% to 2% acetic acid solution delivered rectally. This experiment found that Nerium oleander leaf extract aids recovery in mice with UC, suggesting its potential for future therapeutic use in UC patients and drug design.

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