JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/49705
RESEARCH ARTICLE

Effectiveness of Herbal Syrup in Treating Chemical Induced Ulcerative Colitis

Rohit Agrahari*, Guno Sindhu Chakraborthy and Sayan Chatterjee

Department of Pharmacology, Faculty of pharmacy, Parul Institute of Pharmacy and Research, Parul University, Vadodara - 391760, Gujarat, India; g.chakraborthy19159@paruluniversity.ac.in

Abstract

Background: Ulcerative Colitis (UC) is a prevalent disease that can lead to serious medical conditions, including death. Ulcerative colitis and autoimmune disease are related to each other as this disease share some common pathways, resulting can lead to some other medical conditions like serious gastrointestinal disease tumour such as growth of the cancerous cell in the colon often the result is caused the chronic inflammation in the colon. Strikingly, this disease can put life at risk multiple factors are responsible for the outcome or progression of the ulcerative colitis or autoimmune disease. Now days the modern medicine is very effective and potent as controlling the progression of the Ulcerative Colitis (UC) and autoimmune disease but comes with a higher price tag of side effects. **Aim:** The modern medication needs to be administered continuously and in the higher doses which lead to negative drawback. Hence, in this study we optioned for the herbal formulation which will be at low cost and no side effect and also has broad therapeutic range, so it can be used at high dose also. **Methods:** We used the herbal formulation for 28 days in order to treat the disease condition like UC and it was observed that the herbal formulation was capable to treat disease conditions. The high Tumour Necrosis Factor TNF-alpha and IL-6 was brought back to normal level using the herbal formulations as treatment and no side effect was observed. **Results:** The results of the whole investigation demonstrated that combinational therapy is more effective and has fewer side effects than the high dosage of medication presently used in normal treatment. **Conclusion:** Combinational therapy has the potential to be more preclinical significant and more utilised in the future than the present therapeutic approach.

Major Finding: The study demonstrated that a 28-day treatment with the herbal formulation effectively ameliorated ulcerative colitis by significantly reducing elevated TNF-α and IL-6 levels to near-normal values, without observable side effects. These findings suggest that combinational therapy incorporating herbal formulations may offer a safer and more effective alternative to high-dose conventional medications.

Keyword: Autoimmune Disease, Bilirubin, IBD, IL-6, TNF-alpha, Ulcerative Colitis

1. Introduction

Inflammatory Bowel Disease (IBD) is a chronic colon disease. The pathology of IBD is still elusive in nature. It causing autoimmune disease such as rheumatic arthritis, thyroids, vitiligo, Type 1 diabetes, multiple sclerosis and myasthenia gravis. It is divided into 2 parts 1st is Chron's disease and 2nd is Ulcerative colitis. Chron's disease is a part of the IBD in which the digestive tract becomes swollen and irritated. The most common part affected is small intestine and starting from the large intestine part. The Ulcerative colitis is also a part of IBD, the chronic inflammation bowel disease that can cause the ulceration in the colon as well as rectum part of the intestine[1]. Factors involved in inducing IBD include like genetic, environmental, microbial or immunological factors. The symptoms of IBD are diarrhea, constipation, abdominal pain, vomiting, cramping in lower abdominal, bloody fecal. The marker of the ulcerative colitis is Myeloperoxidase activity (MPO), bilirubin level, Calprotectin, IL-6, TNF-alpha, DAI index and C - reactive protein.

*Author for correspondence

Article Received on: 05.05.2025 Revised on: 06.07.2025 Accepted on: 30.07.2025

The glucocorticoids are playing a crucial role in the treatment of IBD but there are a lot of side effect such as cardiovascular disease, weakness and other effects. The plant extract plays a crucial role in the treatment of the inflammation in the colon or decrease the effect of the Ulcerative colitis. They don't show major side effect in human body as well as animal body because they have a lot of an antioxidant activity, anti-inflammatory activity and immunomodulatory activity[2]. *Psoralia corylifolia,* is having the anti-inflammatory activity, immunomodulatory activity, antibacterial, antifungal, antioxidant, and antitumor properties *Nigella sativa* is having the antioxidant, anti-inflammatory, immunomodulatory, anticancer, antidiabetic, and cardiovascular activity that help in treating the inflammation as well as protecting from the cause of autoimmune disease.

2. Correlation between Inflammatory Bowel Disease and Autoimmune Disease

The pathophysiology of the Inflammatory Bowel disease is not fully known but its cause by the autoimmune mechanism is uncertain. There are many cases reported in the recent days that are appeared to be Inflammatory Bowel disease by a various type of disorder that are autoimmune based, like Thyroid disease, Rheumatic arteritis, Haemolytic anaemia[3]. It may be caused due to Auto-immune reaction, the autoimmune disorder is a part of a broad spectrum of a disease, and these are cause for the auto anti-bodies. There are finding of the anti-bodies in colon Ulcerative Colitis (UC) has been confirmed by some work but not by the other microbe's contamination, in ulcerative colitis (UC), colonic tissue extracts serve as antigens for detecting colon-specific antibodies. Specific fluorescent antibody assays reveal that sera from UC patients react with cytoplasmic antigens of colonic epithelial cells. Clinical and immunological evidence suggests that UC is frequently accompanied by autoimmune disturbances, with comorbidities such as systemic lupus erythematosus and other chronic inflammatory autoimmune disorders. The UC is not a single disease it is syndrome with different disease caused through auto immune. There are some evidences that the patients suffering from the UC are also having a Rheumatic Arteritis (RA)[4]. According to Saarinen *et al*; 24 % of the UC patients have at least one autoimmune disease. Autoimmune antibody presence, a key immunological feature of inflammatory bowel disease (IBD), is observed in 70–80% of ulcerative colitis (UC) patients who present with concomitant autoimmune disorders, rather than isolated UC. The herbal formulation has worked to reduce inflammatory mediators by inhibiting the TNF-alpha not binding on the TNF-alpha 1 receptor and other inflammatory mediators are not binding their affinity site. When they did not bind to the receptor they did not show any inflammation. It acts on the NF-κB signalling pathways and inhibit the inflammation[5].

3. Materials and Methods

3.1 Drug Authentication and Extraction

The seeds of both the plant *P. corylifolia, N. sativa* were obtained from the *Ayurveda* Department of Parul Institute of *Ayurveda*, Parul University, Vadodara, and Gujarat. They were authenticated and voucher specimens are preserved in the department for further use.

3.2 Herbs Collection and Extraction

The seeds were weighted in equal quantity (100gm each) to a total weight of 200gm. Further from it 100gm were taken and it was pulverized by using a mechanical device. The powder was sieved through sieve no 40 to obtain fine powder which was further taken up for maceration process with 50:50 ratio of Water and Alcohol (Hydro alcoholic) and kept in dark for 7 days with occasional stirring. After 7 days it was filter and the filtrate was concentrated over rotary evaporator at 40° C to obtain a semi-solid mass. It was kept for drying and further used for experimental procedures.

3.3 Preparation of the Herbal Formulation (Syrup)

The concentrated extract was taken 0.125g in hydro alcoholic solution (50:50) ratio and we have taken the Acacia 2% of the drug weight 0.025g soaked with in 10 ml of distilled water for 30 min. Then it was dissolved in the hydro alcoholic solution. Sodium benzoate (0.2 g) was dissolved in the same hydro alcoholic solution. Separately, 3.5 g of sorbitol was dissolved in lukewarm

distilled water with continuous stirring. This sorbitol solution was mixed into the hydro alcoholic solution; the final volume was made up with distilled water[6].

3.4 Animals

Male Wistar rat aged 9-11 weeks (150-200 g) were obtained from Sync Bio Research Ltd, Ahmedabad, Gujarat, India after approval from Institutional Animals Ethics Committee (IAEC), Parul Institute of Pharmacy and Research, Parul University (Approval No.: PIPR984/2024/02/09). All experimental protocols were conducted according to the guideline of Committee for Control and Supervision of Experiments on Animals (CCSEA), India. Rats were maintained at a constant temperature of 21 ± 10° C and humidity of 50 ± 10% (12hrs day-night cycle). They were housed in groups of 4 animals per cage with free access to water or standard commercial food (*ad libitium*) throughout the experiment.

4. Methods

4.1 *In Vivo* Study

4.1.1 Induction of Ulcerative Colitis

Rat was randomly selected divided in 5 groups (Control group, Standard group, Disease Control group, Test I low dose , Test II high dose) (n=6) each group with 6 animals were administered with per oral saline (10mg/kg), (10mg/kg) Sulfasalazine (500mg/kg) Herbal formulation 50mg/kg, 200mg/kg from day 0 to 7. Induction of colitis through intrarectal route was carried out on day 4 with 4% of acetic acid by poly urethane tube. The animals were hold for 30 to 60 sec for prevention of any leakage by the inducer acetic acid[7]. The dose selection was according to the LD_{50} of crude drug.

4.2 Evaluation Parameter

Evaluation parameters were selected on the basis of literature review like body weight, antioxidant activity, hematology parameters, inflammation mediator like TNF-α and IL-6 that are responsible for the inflammation, colon weight, DAI index for the mentioned disease.

4.3 Assessment of Body Weight

After and before induction of the UC in rat, weigh all the animals after the induction of UC. The weight of the rat's decreases day by day due to diarrhea, bloody diarrhea, fecal, abdominal pain (Figure 1)[8].

4.4 Antioxidant Activity (SOD)

The antioxidant activity was determined as describe by Misra and Fridovich methods. The rats are taken and given the anesthesia I.P, after they are anesthetized the rats are scarified and collected the colon tissue and then wash with ice-cold water and homogenate the tissue or centrifuged at 2000 rpm for 20 min at 25° C. After homogenate the supernatant are collected and mix 1ml of Carbonate bicarbonate buffer (0.1M and PH 9.2). After addition the reaction was started and 50ul of adrenalin was added, then the absorbance was measure against a blank at 480nm for 4 min using UV- visible spectroscopy meters (Figures 3)[9].

4.5 Malondialdehyde (MDA)

The level of Malondialdehyde estimated as describe by health and parker. Tissue excreted of 1 ml was added to 3 ml of mixture containing 20 % TCA (Trichloroacetic Acid) or 0.5% (TBA) Thiobarbiturate Acidafter, the added mixture was heated at 95° C for 30 min and cooled in ice-bath, further centrifuged at 20000 rpm for 10min. After centrifugation the absorbance of the MDA- TBA complex was measured at 532 nm against blank by using ultra violet spectrophotometer. The concentration (nmol/mg protein) of MDA was calculated by using MDA estimation coefficient $.56\times10^{-5}m^{-1}cm^{-1}$ (Figure 2)[10].

4.6 Estimation of Serum TNF Alpha, IL-6 and CRP

The UC was induced in rats, on 8th day blood sample was collected in activated tube and it was centrifuged at 3000 rpm for 30 min at 4° C to obtain the serum. The collected serum was estimated for TNF alpha, Il-6 and CRP by Pivokine ELISA Kits (Figures 5, 6 and 7)[11].

4.7 Hematology Parameters

Blood sample was collected into sterile tube with EDTA for prevention of coagulation and complete blood count was carried out in automated cell analysis (Table 1)[12].

4.8 Colon Weight

The rats were given the anesthesia by I.P xylazine and after anesthetized the animals are sacrificed and

collected the colon. After the colon was cleaned with ice-cold water, after cleaning the colon was weighed. The normal colon weight was 1.2 gm but the disease control colon weight was 2.8 gm. Further the colon length was measured with the help of scale (Figure 8)[13].

4.9 Measurement of Bilirubin

To measure the bilirubin level in blood the Diazo reagent was used. The reagent is prepared through mixing of the sulfanilic acid or sodium nitrite. For the direct bilirubin measurement, take 0.5 ml of blood sample and is mixed with 1 ml of the Diazo reagent and allowed to react for 5 min at room temperature. After 5 min absorbance is measured at the 540nm and 600nm in the spectrophotometer. For the total bilirubin measurement take 0.5ml of blood sample and mixed with 1ml of the caffeine-benzoate reagent which can helps to dissociate the bilirubin from the albumin then add 1ml of diazo reagent and leave for 5min in the room temperature and after 5min add 1ml of ascorbic acid to stop the reaction and 1ml of the alkaline tartrate solution is added to increase the colour development before measuring the absorbance at 600 nm. The indirect bilirubin is calculated by subtracting the direct bilirubin from the total bilirubin. A standard bilirubin solution is used to create a calibration curve for the quantification (Figure 4)[14].

5. DAI Index (Disease Activity Index)

The procedure for calculating the DAI include the different parameters to quantify the disease severity. The symptoms such as stool frequency, rectal bleeding, weight loss and assessed the numerical scores based on the severity. Mild, moderate, severe mild is 1, moderate is 2, severe is 3, that is denoted the disease score in the IBD (Tables 2 and 3)[11].

5. Results and Discussion

5.1 Body Weight

Effect of the herbal formulation on the body weights of acetic acid induced ulcerative colitis in rats. Rats were administered daily the oral treatment of either normal saline (10ml/kg), sulfasalazine (500mg/kg) or Herbal Formulation (HF) at dose of (50mg/kg and 200mg/kg). On the 4th day, administrated the 4% of 2ml of acetic acid through the intrarectal by the polyurethane tube. Changes in body weight were recorded and the data are presented as mean ± SEM (n=6). Statistical analysis was performed by using the one-way ANOVA followed by the Dunnett's test. A significant level of ****P<0.0001 was observed.

5.2 MDA Activity

The effect of the herbal formulation on malondialdehyde (MDA) levels in the colon was evaluated in a rat

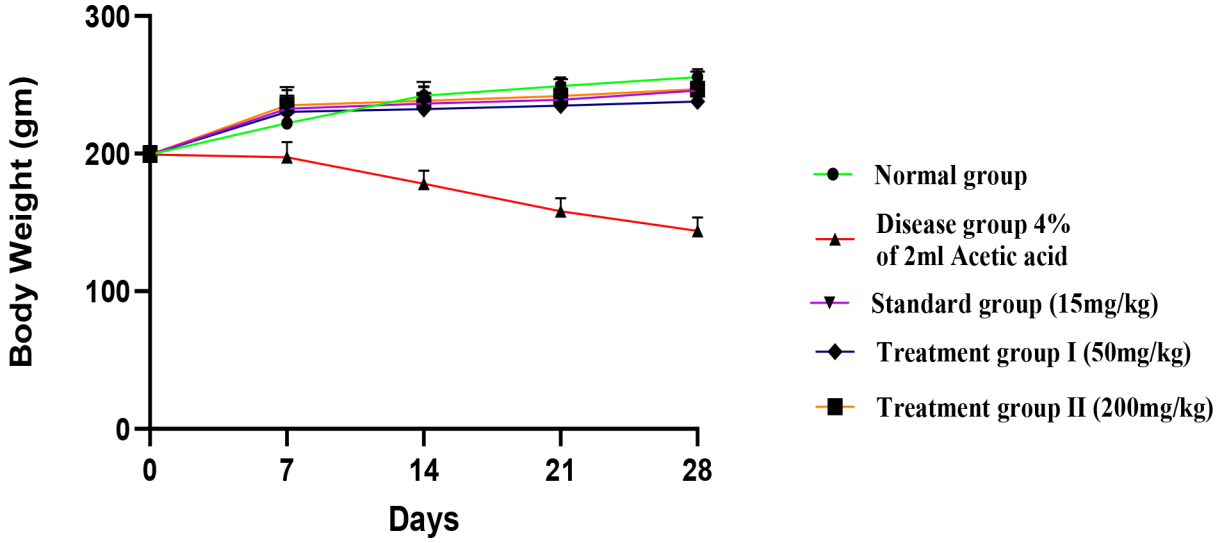


Figure 1. Body weights of animals 0-28 days.

model of colonic injury induced by intrarectal administration of acetic acid. Rats were administered daily oral treatment of either normal saline (10ml/kg), sulfasalazine (500mg/kg) or Herbal Formulation (HF) at dose of (50mg/kg and 200mg/kg). On the 4th day, it was administrated the 4% of 2ml of acetic acid through the intrarectal by the polyurethane tube. On the 28th day the rat was scarified and colons were collected, homogenized or centrifuged. The resulting supernatant was used to assess malondialdehyde MDA activity as a marker of lipid peroxidation. Result was expressed as Mean ± SEM (n=6) and statistically was performed using student's t test. Comparisons were made between the normal control and disease control (****P<0.0001), normal control and standard treatment group (***p<0.001), and normal control and HF 50mg/kg and 200mg/kg group (***p<0.001).

5.3 SOD Antioxidant Activity

Effect of herbal formulation on SOD on the colon inducing the acetic acid through intrarectal route and challenged rat's colon. Rats were administered daily oral treatment of either normal saline (10ml/kg), sulfasalazine (500mg/kg) or Herbal Formulation (HF) at dose of (50mg/kg and 200mg/kg). On the 4th days was administrated the 4% of 2ml of acetic acid through the intrarectal by the polyurethane tube. On the 28th days the rat was scarified and colons were collected, homogenized or centrifuged. The resulting supernatant was used to assess SOD activity as a marker of lipid peroxidation. Result was expressed as Mean ± SEM (n=6) and statistically was performed using student's t test. Comparisons were made between the normal control and disease control (****P<0.0001), normal control and standard treatment group (***p<0.001), and normal control and HF 50mg/kg and 200mg/kg group (***p<0.001).

5.4 Measurement of Total Bilirubin Level

Effect of herbal formulation on serum total level of bilirubin inducing the acetic acid through intrarectal route and challenged rat's colon. Rats were administered daily oral treatment of either normal saline (10ml/kg), sulfasalazine (500mg/kg) or Herbal Formulation (HF) at dose of (50mg/kg and 200mg/kg) daily for 28 days. On the 4th days were administrated the 4% of 2 ml of acetic acid through the intrarectal by the polyurethane tube. On the 7th days, 14th, 21th, 28th the collected blood and serum total level of Bilirubin were assessed by Diazo method. Results were presented as Mean ±SEM (n=6). Statistical analysis was performed by using the one-way ANOVA followed by the Dennett's test. A significant level of ****P<0.0001 was observed.

5.5 Haematological Parameters

Haematological parameters serve as critical biomarkers reflecting physiological and pathological states of the body. They provide essential insights into blood composition, immune function, and overall systemic health.

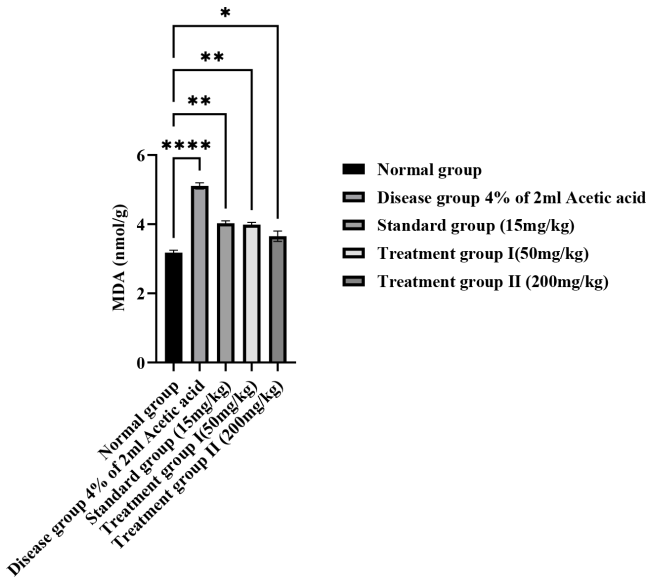


Figure 2. MDA levels in colon of animals.

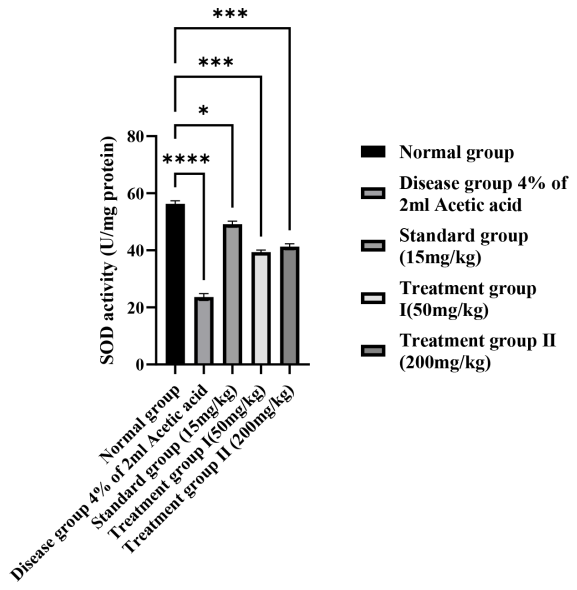


Figure 3. SOD levels in colon animals.

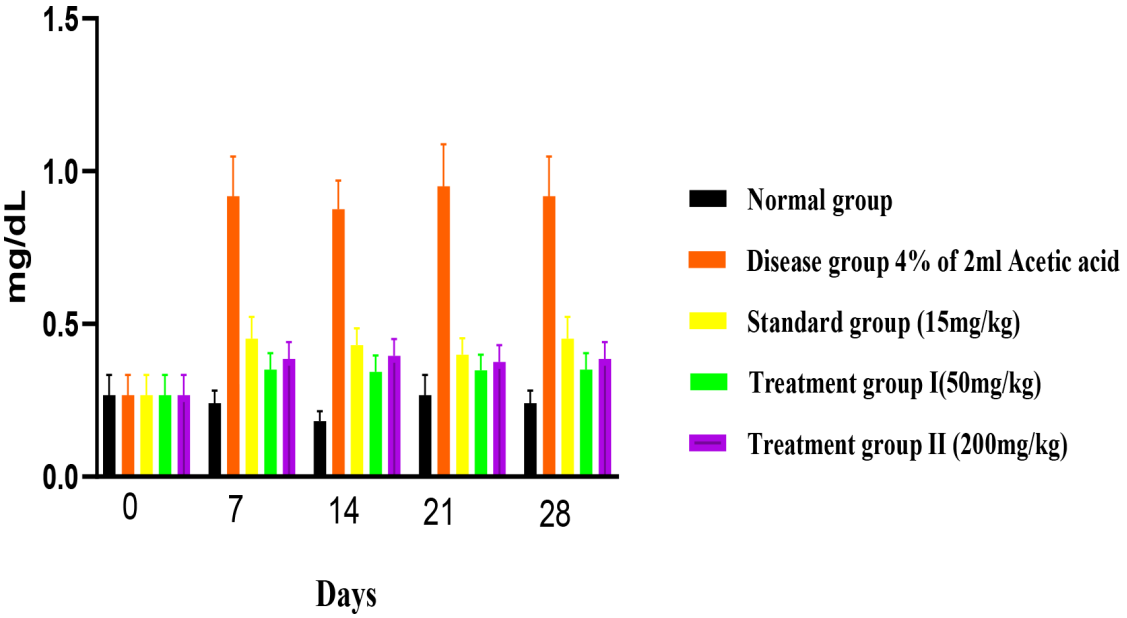


Figure 4. Measurement of total bilirubin levels of animals 0-28 days.

Table 1. Haematological parameter of animals

Treatment Group	WBC (x103/ul)	RBC (x106/ul)	HGB (g/dl)	LYMP (%)	NEUT (%)	HCT (%)	PLT (%103/g)
Normal Group	11.32±1.03	10.56±0.95	14.51±0.77	695±3.33	7.5±0.57	45.72±4.32	593±14.38
Disease Group	13.47±0.97****	8.87±1.18****	13.61±0.89****	57.8±0.89****	8.9±1.12****	42.4±1.80****	631±20.01****
Standard Group	12.71±1.02****	6.95±0.88****	12.92±1.25****	51.9±2.96****	6.7±0.69****	49.31±2.89****	583±36.88****
Test I 50 mg/kg	10.32±1.07****	7.46±0.64****	11.95±0.86****	61.7±6.26****	7.3±0.45****	48.66±4.58****	667±20.77****
Test II 200mg/ kg	12.96±1.06****	7.22±1.22****	13.12±1.86****	52.7±3.12****	7.8±1.7****	50.12±3.7****	632±37.22****

Data were expressed as Mean ±SEM (n=6), statistical analysis was performed using one-way ANOVA followed by Dennett's test. A value of $P<$ 0.0001 was considered highly significant compared to the treated group.

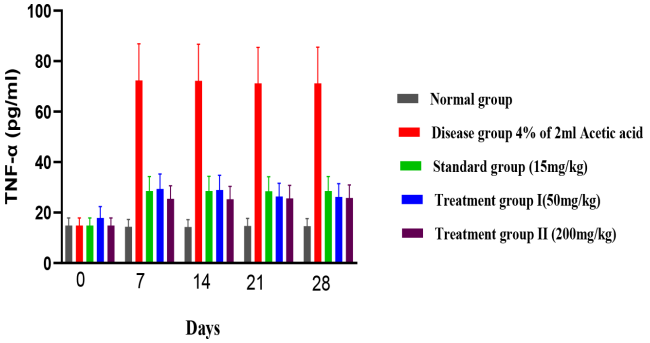


Figure 5. Measurment of TNF-alpha levels of animals 0-28 days.

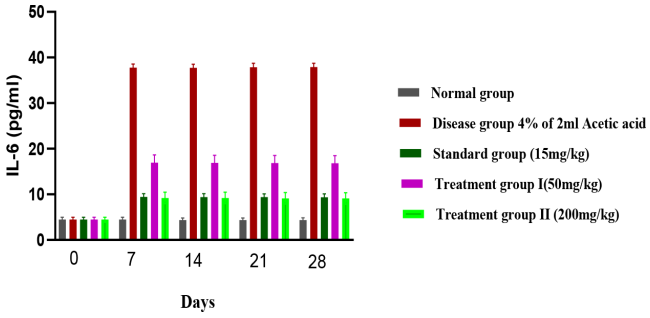


Figure 6. Measurement of IL-6 levels of animals 0-28 days.

5.6 TNF-alpha

Effect of herbal formulation on serum level of TNF-alpha inducing the acetic acid through intrarectal route and challenged rat's colon. Rats were administered with daily oral treatment of either normal saline (10ml/kg), sulfasalazine (500mg/kg) or Herbal Formulation (HF) at dose of (50mg/kg and 200mg/kg) daily for 28 days. On the 4th days were administrated the 4% of 2ml of acetic acid through the intrarectal by the polyurethane tube. On the 7th day, 14^{th,} 21th, 28th day collected the blood and serum level of TNF-alpha were assessed by ELISA kit. Results were presented as Mean ±SEM (n=6). Statistical analysis was performed by using the one-way ANOVA followed by the Dennett's test. A significant level of ****P<0.0001 was observed.

5.7 IL-6 (Interleukins- 6)

Effect of herbal formulation on IL-6 inducing the acetic acid through intrarectal route and challenged rat's colon. Rats were administered daily oral treatment of either normal saline (10ml/kg), sulfasalazine (500mg/kg) or Herbal Formulation (HF) at dose of (50mg/kg and 200mg/kg) daily for 28 days. On the 4th days were administrated the 4% of 2ml of acetic acid through the intrarectal by the polyurethane tube. On the 7th day, 14^{th,} 21th, 28th day collected the blood and serum level of IL-6 was assessed by ELISA kit. Results were presented as Mean ±SEM (n=6). Statistical analysis was performed by using the one-way ANOVA followed by the Dennett's test. A significant level of ****P<0.0001 was observed.

5.8 CRP level

Effect of herbal formulation on serum level of CRP inducing the acetic acid through intrarectal route and challenged rat's colon. Rats were administered daily oral treatment of either normal saline (10ml/kg), sulfasalazine (500mg/kg) or Herbal Formulation (HF) at dose of (50mg/kg and 200mg/kg) daily for 28 days. On the 4th day were administrated the 4% of 2ml of acetic acid through the intrarectal by the polyurethane tube. On the 7th day, 14^{th,} 21th, 28th day collected the blood and serum level of CRP was assessed by Immunoturbidimetry. Results were presented as Mean ±SEM (n=6). Statistical analysis was performed by using the one-way ANOVA followed by the Dunnett's test. A significant level of ****P<0.0001 was observed.

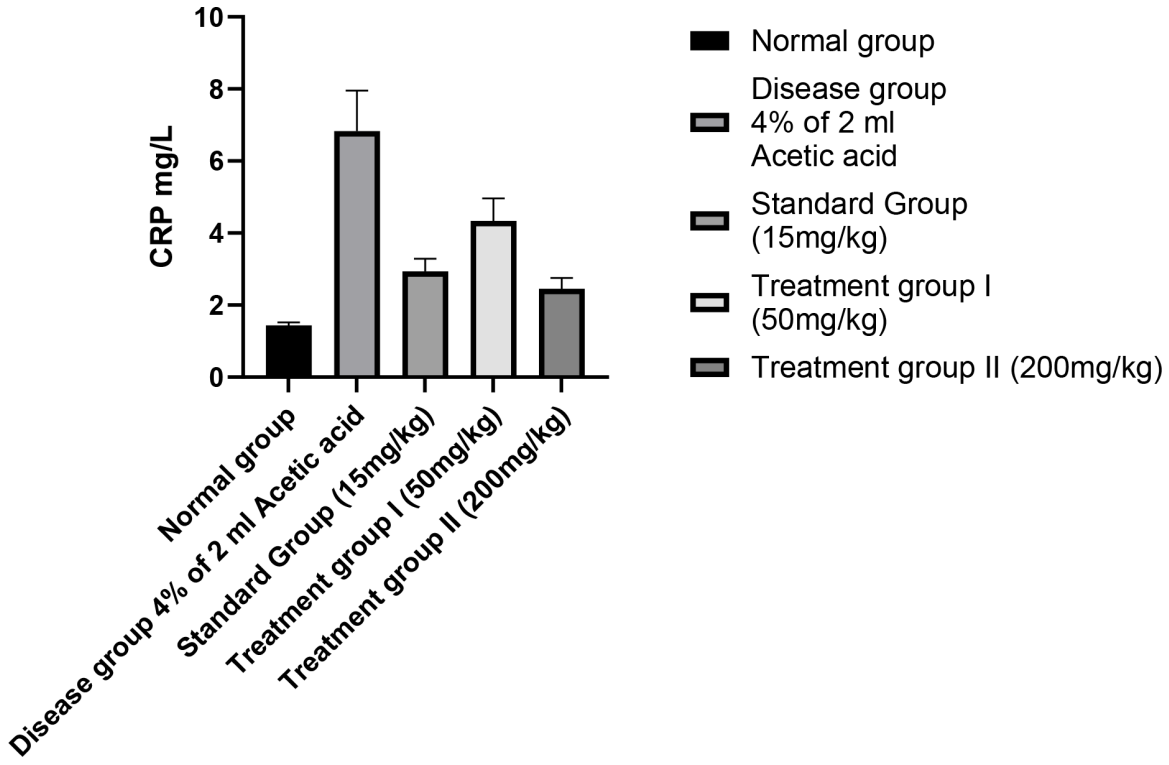


Figure 7. Measurement of CRP levels of animals 0-28 days.

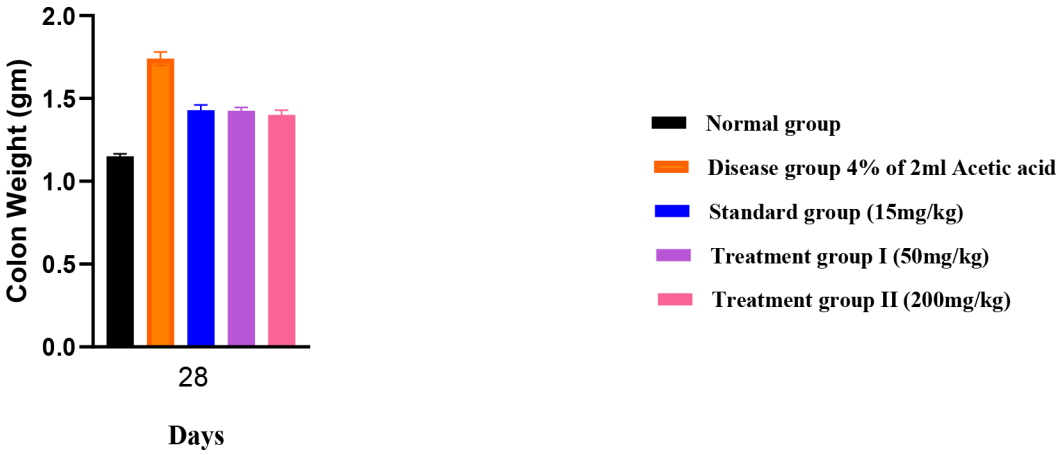


Figure 8. Measurement of colon weight of animals on 28 days.

Table 2. Disease index of ulcerative colitis

Animal Group	Body Weight	% Decrease of Body Weight	Stool Consistency	Rectal Bleeding	DAI Score
Normal	204.17	0.00	Normal	Normal	0.00
Acetic Acid	172.67	15.43	Bloody Diarrheal	Occult^{+++}	3.79
Standard Drug	186.50	8.65	Loose Stool	Occult^{+}	1.62
Test I 50mg/kg	182.50	5.65	Loose Stool	Occult^{+}	0.9
Test II 200mg/kg	190.83	6.53	Loose Stool	Occult^{+}	0.06

Table 3. Disease Activity Index (DAI) scoring parameters in experimental model

Score	Body Weight Decrease	Stool Consistency	Rectal Bleeding
0	<1	Normal	Normal
1	1-5		
2	5-10	Loose Stools	
3	10-20		
4	>20	Diarrheal	Gross Bleeding

5.9 Colon Weight

Effect of the herbal formulation on the colon weight acetic acid induced ulcerative colitis in rats. Colitis was induced through the acetic acid by the intrarectal route 4% of 2ml. Rat was sacrificed on the 28 days and colon weights were assessed. Results were presented as Mean ±SEM (n=6). Statistical analysis was performed by using the one-way ANOVA followed by the Dunnett's test. A significant level of ****P<0.0001 was observed.

6. DAI (Diseases Index)

Effect of herbal formulation on macroscopic score of colons acetic acid- induced colitis in rats. Colitis was induced in rat by intrarectal administration of 2ml of 4% acetic acid on the 28th the rat was euthanized and their colon were excised longitudinally opened and washed, examined. The microscopic damage of the colon was scored of 0 to 100% with ascending score reflecting greater severity of inflammation, results were presented as % change in the body weight, stool consistency, rectal bleeding, DAI score.

6. Discussion

The animal groups (II-IV) were given the dose of the acetic acid 4% to 2ml for the induction of the disease that can increase the TNF-alpha, IL-6, bilirubin level and elevate the TNF -alpha level in the blood. Group I was kept the Normal group for comparing

the parameters with the other groups. After 7 days of Acetic acid dose animal blood was collected from each group to check all the needed parameters to confirm the induction of the Ulcerative Colitis. According to the result, animals of the group II, III, IV and V were ulcerative colitis and also their blood level TNF-alpha, IL-6 was also high as compared to the normal group. Standard group (III) animals were given Basalazide (15mg/kg) orally on a daily basis for 28 days of period and the Treatment group (IV) and (V) animals were given the low and high dose, Group IV were given the low dose (50mg/kg) and V group were (200mg/kg) dose of the herbal formulation (syrup form) orally on a daily basis for 28 days of period. Blood was collected from all group at an interval of 7 days from the starting day of treatment to check the parameter like TNF-alpha, IL-6, body weight, bilirubin level and the result shows that TNF-alpha, IL-6 is high in the initial time but with the treatment TNF-alpha, IL-6 was back to normal level after the 28 days and it was gradually decreasing with treatment both in standard (III) and treatment (IV) and (V) group and same was observed with bilirubin level and body weight decrease in initial stage but after 28 days the increase the normal body weight. Increased with the treatment both in the standard and treatment group. The disease control group showed the increased level of TNF-alpha, IL-6, bilirubin level and decrease the body weight without any treatment. Hence it can be said that the herbal formulation was as effective as the standard treatment as the treatment group animals were back the normal conditions from the Ulcerative colitis after using the herbal formulation as and previously this formulation was never used in the treatment of the ulcerative colitis and autoimmune disease. Though the individual herbs was used to treat that disease now these combinations is newly used for the treatment and this combination showed synergistic activity in the treatment of the disease.

7. Conclusion

From this study it was concluded that the prepared herbal formulation was effective in reducing the biochemical parameters when compared with the standard group of animals. The research can be further investigated for exploring in more depth for treating the disease Ulcerative colitis with a synergistic effect of auto immune disorder which will be a boon to mankind.

8. Acknowledgment

We would like to acknowledge Parul Sevasharm Hospital for performing the ELISA on IL-6, TNF-alpha, and CBC.

9. Author Contribution

Rohit Agrahari, G S Chakraborthy and Sayan Chatterjee design the study. Rohit Agrahari conducted all the experimental and evaluation parameters including *in-vivo* study. G S Chakraborthy was in the preparation of manuscript and its alignment with the findings. Sayan Chatterjee had looked in to the literature writing content. All authors have read and agreed to the published version of the manuscript.

10. References

1. Bengi G, Cıvak M, Akarsu M, Soyturk M, Ellidokuz E, Topalak O, *et al*. Prevalance of Celiac disease in patients with inflammatory bowel disease in Turkish population. Gastroenterol Res Pract. 2019. https://doi.org/10.1155/2019/6272098
2. Rosendorff C, Grieve NW. Ulcerative oesophagitis in association with ulcerative colitis. Gut. 1967; 8(4):344-347. https://doi.org/10.1136/gut.8.4.344
3. Wright R, Truelove SC. Auto-immune reactions in ulcerative colitis. Gut. 1966; 7(1):32-40. https://doi.org/10.1136/gut.7.1.32
4. Stevens HP, Ostlere LS, Rustin MHA. Systemic lupus erythematosus in association with ulcerative colitis: Related autoimmune diseases. Br J Dermatol. 1994; 130(3):385-389. https://doi.org/10.1111/j.1365-2133.1994.tb02938.x
5. Charpentier CG, Champigneulle J, Williet N, Biroulet LP, Morali A. The association of autoimmune diseases with pediatric ulcerative colitis does not influence its disease course. Scand J Gastroenterol. 2016; 51(1):33-40. https://doi.org/10.3109/00365521.2015.1058415
6. Patil JK, Mali DR, Jain SM. Formulation and evaluation of herbal syrup. World J Pharm Res. 2022; 8(6):1061-1067.
7. Rejaie SSA, Abuohashish HM, Enazi MMA, *et al*. Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats. World J Gastroenterol. 2013; 19(34):5633-5644. https://doi.org/10.3748/wjg.v19.i34.5633
8. Akil DS, Leifeld L, Pfutzer R, Morgenstern J, Kruis W. The effect of body weight on the severity and clinical course of

ulcerative colitis. Int J Colorectal Dis. 2015; 30(2):237-242. https://doi.org/10.1007/s00384-014-2051-3

9. Rana SV, Sharma S, Prasad KK, Sinha SK, Singh K. Role of oxidative stress and antioxidant defence in ulcerative colitis patients from north India. Indian J Med Res. 2014; 139(4):568-571. PMID: 24927343. PMCID: PMC4078495.
10. Duryee MJ, Ahmad R, Eichele DD, Hunter CD, Mitra A, Talmon GA, *et al*. Identification of immunoglobulin G autoantibody against malondialdehyde-acetaldehyde adducts as a novel serological biomarker for ulcerative colitis. Clin Transl Gastroenterol. 2022; 13(4):e00469. https://doi.org/10.14309/ctg.0000000000000469
11. Feng W, Zhu L, Liu Y, Xu L, Shen H. C-reactive protein/albumin ratio and IL-6 are associated with disease activity in patients with ulcerative colitis. J Clin Lab Anal. 2023; 37(3):e24843. https://doi.org/10.1002/jcla.24843
12. Gurler M, Can G, Can H, Tezel HA, Unsal G, Soylu AR, *et al*. The relationship between hematological parameters and disease activity in İnflammatory bowel disease. Anatolian Curr Med J. 2020; 2(3):68-74. https://doi.org/10.38053/acmj.724021
13. Schieffer KM, Bruffy SM, Rauscher R, Koltun WA, Yochum GS, Gallagher CJ. Reduced total serum bilirubin levels are associated with ulcerative colitis. Plos One. 2017; 12(6):e0179267. https://doi.org/10.1371/journal.pone.0179267
14. Walsh AJ, Ghosh A, Brain AO, Buchel O, Burger D, Thomas S, *et al*. Comparing disease activity indices in ulcerative colitis. J Crohns Colitis. 2014; 8(4):318-325. https://doi.org/10.1016/j.crohns.2013.09.010