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Pharmacological Profiling and Therapeutic Evaluation of Herbal Plants in Inflammatory Bowel Disease Management

Devidas Naik[1*,] Dr. Vivek Gupta[2]

[1*,2]Department of Pharmaceutical Science, Monad University, Pilkhuwa UP India 245304.

***Corresponding author:** Devidas Naik
*Mail ID: naikdevidas1993@gmail.com

Abstract

The objective of this research is to evaluate the effectiveness of combination therapy involving Trimetazidine with beta blockers, such as Metoprolol, in managing anginal episodes, given the limitations of traditional hemodynamic agents in treating all individuals with anginal problems. While medications like nitrates, beta-adrenergic antagonists, and calcium channel blockers are commonly used to reduce myocardial oxygen demand and alleviate cardiac strain, they often produce undesirable hemodynamic effects such as changes in blood pressure, heart rate, and cardiac output. Consequently, a significant proportion of patients continue to experience chronic angina despite treatment, increasing the risk of future heart issues.

Several clinical studies, including the Total Ischemic Burden European Trial (TIBET), International Multicenter Angina Exercise (IMAGE) study, Combination of Trimetazidine with Metoprolol in stable effort Angina, Poland (TRIMPOL II) study, and Trimetazidine in Angina Combination Therapy (TACT) study, have reported conflicting findings regarding the efficacy of combination therapy with two hemodynamic agents. However, previous research suggests that combining Trimetazidine with beta blockers or long-acting nitrates can lead to significant improvements in exercise stress test parameters and better management of uncontrolled anginal symptoms, with good patient tolerability.

This study aims to explore the potential of metabolic therapy, exemplified by the combination of Trimetazidine with Metoprolol, as a contemporary approach to ischemic illnesses. The lack of unique dosage forms and comprehensive research investigations underpinning this combination make them promising candidates for further investigation in this study.

Keywords: Inflammatory Bowel Disease; Herbal Plants; Pharmacological Profiling; Therapeutic Evaluation; Management

1.Introduction

Inflammatory Bowel Disease (IBD), comprising Crohn's disease and ulcerative colitis, is a chronic inflammatory disorder of the gastrointestinal tract, characterized by unpredictable bouts of inflammation and remission. Conventional pharmacological therapies for IBD, including immunosuppressants and anti-inflammatory drugs, often exhibit limited efficacy and are associated with adverse effects. Consequently, there is a pressing need to explore alternative treatment modalities with improved efficacy and safety profiles [1].

The use of herbal plants as therapeutic agents in IBD management has garnered increasing attention due to their rich pharmacological properties and potential to modulate the underlying inflammatory processes. Herbal remedies, derived from various plant sources, contain a plethora of bioactive compounds, including polyphenols, flavonoids, terpenoids, and alkaloids, which exert diverse pharmacological effects, including anti-inflammatory, antioxidant, and immunomodulatory actions [2].

The rationale behind investigating herbal plants for IBD management lies in their ability to target multiple pathophysiological pathways implicated in the disease process. These pathways include the dysregulation of immune responses, disruption of intestinal barrier function, and oxidative stress-mediated tissue damage. By modulating these underlying mechanisms, herbal plants hold promise as adjunctive therapies for IBD, capable of mitigating inflammation, promoting mucosal healing, and improving overall disease outcomes [3].

Moreover, herbal remedies offer several advantages over conventional pharmacological agents, including a lower risk of adverse effects, better tolerability, and potentially lower costs. Additionally, the complexity of herbal formulations allows for the synergistic interaction of multiple bioactive compounds, which may enhance therapeutic efficacy and reduce the likelihood of drug resistance [4.6].

In light of these considerations, this research article aims to systematically evaluate the pharmacological profile and therapeutic potential of selected herbal plants in the management of IBD. Through a comprehensive review of preclinical and clinical studies, we seek to elucidate the underlying mechanisms of action of herbal remedies and identify promising

candidates for further investigation and clinical development. By advancing our understanding of the role of herbal plants in IBD management, we aim to contribute to the development of evidence-based therapeutic strategies that improve patient outcomes and quality of life [7,8].

2. Material and Method

2.1 Plant Material Collection and Authentication

The tubers of Cyperus rotundus and the stem bark of Holarrhena antidysenterica were procured from a reputable wholesaler located in Ahmedabad. The authenticity of the plant material was ensured through rigorous examination of their morphological characteristics. Authentication of the plant samples was conducted by Dr. Geetha, Senior Scientist, and Dr. Satyabrata Maiti, Director, both from the Directorate of Medicinal and Aromatic Plants Research in Boriavi, Anand, Gujarat.

The authentication process involved a thorough assessment of key botanical traits specific to Cyperus rotundus and Holarrhena antidysenterica. These traits included the morphology of leaves, stems, flowers, and other distinguishing features unique to each species. Following the authentication process, voucher specimens of the plant materials were prepared and submitted to the herbarium at Anand Pharmacy College, Anand, with the following reference numbers: APC/2011-2012/SAJ/CR/01 for Cyperus rotundus and APC/2011-2012/SAJ/HA/02 for Holarrhena antidysenterica [9].

2.2 Animal Experimentation and Ethical Approval

Adult male Sprague Dawley (SD) rats, weighing between 250-300g and aged 10-11 weeks, were procured from a certified supplier. Upon arrival, the rats were acclimatized to the laboratory conditions for a period of one week before the commencement of experiments. Throughout the study, the rats were housed in standard laboratory cages with free access to commercial rat chow and water ad libitum. The animal housing facility maintained a controlled environment, with a 12-hour light/dark cycle, ambient temperature set at 22±1°C, and relative humidity maintained at 55%. Ethical approval for all experimental procedures was obtained from the Institutional Animal Ethical Committee of Anand Pharmacy College, in accordance with the guidelines established by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No. 9012 dated 26th Dec 2009).

This comprehensive methodology ensures the standardization and ethical conduct of all animal experiments conducted as part of the study, aligning with internationally recognized guidelines for the humane use of laboratory animals [10-11].

2.3 Successive Solvent Extraction

Dry extracts were obtained from the powdered stem bark of Holarrhena antidysenterica and powdered tubers of Cyperus rotundus using a successive solvent extraction method. The extraction process was conducted in a Soxhlet apparatus for 48 hours, with solvents of increasing polarity. Following each extraction, the solvent was filtered, and the resulting filtrate was evaporated under reduced pressure to obtain the dry extract. Fresh powder was used for each extraction to ensure consistency and prevent contamination between extracts. The extracted materials were stored in a cool, dry location to maintain their stability. For Holarrhena antidysenterica, the successive extraction was performed using petroleum ether (PEHA), hexane (HEHA), chloroform (CHHA), acetone (ACHA), and hydromethanol (MEHA). Similarly, Cyperus rotundus was subjected to successive extraction with petroleum ether (PECR), hexane (HECR), chloroform (CHCR), acetone (ACCR), and hydromethanol (MECR). The choice of solvents was based on their polarity, ensuring the extraction of a wide range of phytoconstituents from the plant materials. This methodological approach, derived from established pharmacognostic texts, facilitated the comprehensive extraction of bioactive compounds from both Holarrhena antidysenterica and Cyperus rotundus for subsequent pharmacological evaluation [12,13].

2.4 Phytochemical Tests

Various phytoconstituents, including alkaloids, carbohydrates, tannins, lipids, oils, steroids, saponins, and flavonoids, were screened for in the different extracts obtained from the stem bark of Holarrhena antidysenterica and tubers of Cyperus rotundus. Alkaloids were detected using Mayer's and Dragendorff's reagents, while the presence of carbohydrates was assessed using Molisch's test. Tannins were identified using ferric chloride solution, and lipids and oils were detected through their characteristic staining with Sudan III solution. Steroids were detected using a specific color reaction with sulfuric acid and acetic anhydride, while the presence of saponins was determined by observing frothing upon shaking with water. Additionally, flavonoids were identified using various chemical tests, including the Shinoda test and the alkaline reagent test. These standardized phytochemical tests were conducted according to established protocols to assess the qualitative presence of bioactive compounds in the extracts, providing valuable insights into their potential pharmacological properties [14,15].

2.5 Grouping and Drug Administration:

The care and handling of the animals adhered to the guidelines outlined in section 3.2.3. An 18-day treatment regimen was implemented, during which twelve groups, each consisting of three randomly selected rats, were utilized. Considering the exploratory nature of the trials exploring the potential benefits of various extracts, three animals per group were deemed appropriate. Throughout the study period, all animals were provided unrestricted access to water and subjected to a 24-hour fasting period preceding the commencement of the experiment [16].

During the 18-day duration of the study, Group I served as the no-intervention control group, while Group II functioned as the control for the induced model of colitis. Animals in Groups III through XII were orally administered with PEHA (600 mg/kg), HEHA (600 mg/kg), CHHA (600 mg/kg), ACHA (600 mg/kg), and MEHA (600 mg/kg) once daily for 18 days. A similar administration protocol was followed for PECR (800 mg/kg), HECR (800 mg/kg), CHCR (800 mg/kg), ACCR (800 mg/kg), and MECR (800 mg/kg) extracts [17-20].

On day 11 of the trial, animals in Groups II through XII were induced with dinitrobenzene sulfonic acid (DNBS) to induce colitis. High doses of both effective herbs were administered to ensure adequate pharmacological response. Subsequent measurements and evaluations were conducted in accordance with the parameters. This rigorous grouping and drug administration protocol enabled the systematic investigation of the therapeutic potential of the herbal extracts in the management of colitis [21,22].

2.6 Subacute Toxicity Studies of MEHA and CHCR

Male and female Sprague Dawley rats weighing between 200-250g were procured from a certified supplier and acclimatized to laboratory conditions for a week before the experiment. They were housed under controlled environmental conditions with standard laboratory chow and water provided ad libitum. The rats were randomly divided into groups, with each group consisting of 5-10 animals per gender. MEHA and CHCR extracts were orally administered to the experimental groups at doses ranging from 100 mg/kg to 2000 mg/kg body weight once daily for 28 days, while a control group received an equivalent volume of the vehicle used for extract preparation [23-25].

Throughout the study period, animals were closely monitored for signs of toxicity, including changes in behavior, food and water consumption, body weight, and mortality. Detailed clinical observations were recorded daily, and body weights were measured weekly. At the end of the treatment period, blood samples were collected for hematological and biochemical analysis, including assessment of hematocrit, hemoglobin concentration, red and white blood cell counts, platelet count, serum levels of liver enzymes (ALT, AST), kidney function markers (urea, creatinine), and electrolytes (sodium, potassium, chloride) [26].

Selected organs were harvested after blood collection for histopathological examination. Tissue sections were stained with hematoxylin and eosin and examined under a light microscope for any signs of organ toxicity or pathological changes. Data collected from the study were analyzed using appropriate statistical methods, and results were expressed as mean ± standard deviation (SD), with statistical significance set at $p < 0.05$. Additionally, all experimental procedures were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India [27,28].

2.7 Main Efficacy Studies

The study utilized the same drugs and chemicals as previously described. Hydromethanolic whole extracts and extracts using different solvents from Holarrhena antidysenterica (MEHA) and Cyperus rotundus (CHCR) were prepared, dried, and stored. Rats were divided into nine groups and administered standard medicine, MEHA, or CHCR extracts orally for 18 days. Colitis was induced in selected groups. Daily monitoring included food and water consumption, body weight, and bowel movements. Blood samples were collected for serum cortisol analysis. Animals were weighed, and colon measurements were taken before sacrifice. Colon macroscopic damage was scored, and tissue homogenate was prepared for biochemical analysis. Parameters such as malondialdehyde (MDA), superoxide dismutase (SOD), and nitric oxide (NO) activity were measured. Histopathological examination of colon tissues was conducted to evaluate the efficacy of the interventions [29-31].

2.8 Analysis of Gene Expression by Quantitative Real-Time PCR (qPCR)

Drugs and Chemicals, Preparation of Extracts, Grouping and Drug Administration, Methodology, and Parameters Observed: In this study, the same drugs and chemicals as described previous section were used. Extracts of Holarrhena antidysenterica (MEHA) and Cyperus rotundus (CHCR) were prepared by drying in methanol and chloroform. Twelve to fourteen-week-old male Sprague Dawley rats (250-300g) were randomly divided into six groups of three animals each. After a 24-hour fasting period with access to water, Group I served as the no-intervention control, while Group II received 50% ethanol intracolonically on day 11. Group III served as the model control, and Groups IV–VI received oral administrations of standard medicine (5-ASA, 100 mg/kg), MEHA (600 mg/kg), and CHCR (800 mg/kg) once daily for 18 days. DNBS was administered to induce colitis in Groups III-VI on day 11. Tissue samples were collected, and total RNA was isolated using a TRI reagent-based procedure. DNase treatment was performed to remove unwanted DNA,

followed by first-strand cDNA synthesis. Real-time PCR reactions were conducted using the Quantitect SYBR Green PCR kit, and primer specificity was confirmed by NCBI blast. Expression levels of target genes were normalized to GAPDH, and fold changes were calculated using the ddCT method [32-35].

3. Result and Discussion

3.1 Plant Material Collection and Authentication:

The tubers of Cyperus rotundus and the stem bark of Holarrhena antidysenterica were obtained from a reliable wholesaler in Ahmedabad. Rigorous examination of their morphological characteristics ensured the authenticity of the plant material. Authentication was conducted by Dr. Geetha, Senior Scientist, and Dr. Satyabrata Maiti, Director, from the Directorate of Medicinal and Aromatic Plants Research in Boriavi, Anand, Gujarat.

The authentication process involved a comprehensive assessment of key botanical traits specific to Cyperus rotundus and Holarrhena antidysenterica, including leaf morphology, stem structure, flower characteristics, and other distinguishing features unique to each species. Following authentication, voucher specimens of the plant materials were meticulously prepared and deposited at the herbarium of Anand Pharmacy College, Anand, with reference numbers APC/2011-2012/SAJ/CR/01 for Cyperus rotundus and APC/2011-2012/SAJ/HA/02 for Holarrhena antidysenterica.

3.2 Phytochemical Tests

The hydromethanolic whole extract of Holarrhena antidysenterica appeared as a brownish, thick, sticky, smooth solid mass, yielding 19.2%w/w. Analysis revealed the presence of saponins, steroids, flavonoids, alkaloids, and tannins in the extract. Similarly, the hydromethanolic whole extract of Cyperus rotundus exhibited a dark brown, viscous mass, with a yield of 8.1%w/w. Phytochemical analysis detected the presence of carbohydrates, steroids, alkaloids, and tannins in the extract.

Table 1: Details of extracts of bark of Holarrhena antidysentery

Solvent	Color of extract	Consistency of extract	% of Dry extract (%w/w)	Main constituents present
Petroleum ether	Dark yellow	Oily thick residue	0.36	Steroids, fats
Hexane	Dark yellow	Oily thick residue	0.2	Steroids, fats
Chloroform	Greenish brown	Sticky thick mass	0.32	Steroids, alkaloids, saponins
Acetone	Dark green	Sticky thick mass	0.36	Alkaloids, tannins
Methanol-Water	Brown	Sticky smooth solid residue	16.96	Flavonoids, alkaloids, tannins

3.3 Acute toxicity studies of WMEHA and WMECR

The acute toxicity studies of WMEHA and WMECR revealed that the maximum tolerated doses for the hydromethanolic extracts of Cyperus rotundus and Holarrhena antidyseneterica were in the range of 1500 to 2000 milligrams per kilogram, respectively (Sharma et al., 2005). In the context of inflammatory bowel illness induced by DNBS in rats, the effects of WMEHA and WMECR on stool consistency were investigated. The average stool consistency score significantly increased to 1.667±0.333 ($P<0.001$) in the model control group upon intracolonic administration of DNBS, compared to the score of 0.0±0.0 ($P<0.05$) observed in normal control animals. However, vehicle control did not significantly alter stool consistency compared to normal control animals. Treatment with 5-ASA (100 mg/kg, p.o. for 18 days) significantly reduced stool consistency to 0.0±0.0 ($P<0.001$) compared to DNBS control animals. Moreover, treatment with WMEHA (200, 400, and 600 mg/kg, p.o. for 18 days) and WMECR (300, 500, and 800 mg/kg, p.o. for 18 days) also significantly decreased stool consistency scores (0.33±0.33; $P<0.05$, 0.33±0.33; $P<0.05$, 0.33±0.33; $P<0.05$, 0.33±0.33; $P<0.05$, 0.33±0.33; $P<0.05$, and 0.0±0.0; $P<0.001$, respectively) compared to DNBS control animals (Figure 4.1). Statistical analysis was conducted using the Kruskal-Wallis Test followed by the Dunn's post hoc test, where P values <0.05 were considered significant and $P<0.001$ were considered highly significant. Symbols indicating significance levels included #: significantly different from the Model group at $P\leq0.04$, **: significantly different from the Normal group at $P\leq0.001$, and ##: significantly different from the Model group at $P\leq0.001$.

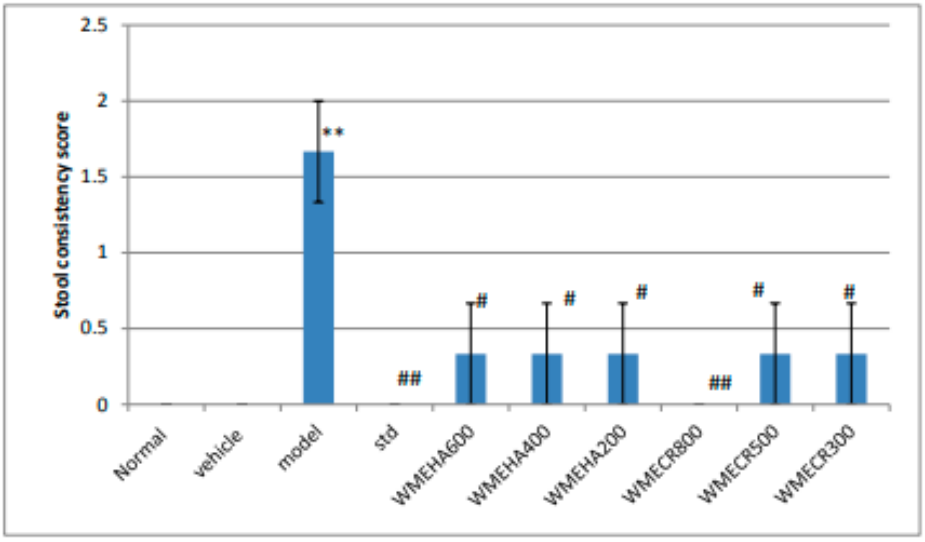

Figure 1: Effect of WMEHA and WMECR on stool consistency in DNBS induced inflammatory bowel disease in rats

Effects of WMEHA and WMECR on changes in wet colon weight/length in DNBS induced inflammatory bowel disease in rats:

The effects of WMEHA and WMECR on changes in wet colon weight/length in DNBS-induced inflammatory bowel disease in rats were investigated. Upon intracolonic administration of DNBS, the wet colon weight/length ratio significantly increased to 0.228±0.005 gm/cm ($P<0.05$) in the model control group compared to normal control animals (0.159±0.006 gm/cm; $P<0.05$). Vehicle control did not significantly alter the wet colon weight/length ratio compared to normal control animals.

Treatment with 5-ASA (100 mg/kg, p.o. for 18 days) significantly decreased the wet colon weight/length ratio to 0.136± 0.001 gm/cm ($P<0.05$) compared to DNBS control animals. Similarly, treatment with WMEHA (200, 400, and 600 mg/kg, p.o. for 18 days) and WMECR (300, 500, and 800 mg/kg, p.o. for 18 days) significantly decreased the wet colon weight/length ratio (0.149±0.008; $P<0.05$, 0.118±0.008; $P<0.05$, 0.123±0.007; $P<0.05$, 0.134±0.013; $P<0.05$, 0.141±0.025; $P<0.05$, and 0.141±0.022; $P<0.05$, respectively) compared to DNBS control animals (Figure 4.2). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test, where P values <0.05 were considered significant and $P<0.001$ were considered highly significant. Symbols indicating significance levels included *: significantly different from the Normal group at $P\leq0.04$ and #: significantly different from the Model group at $P\leq0.04$.

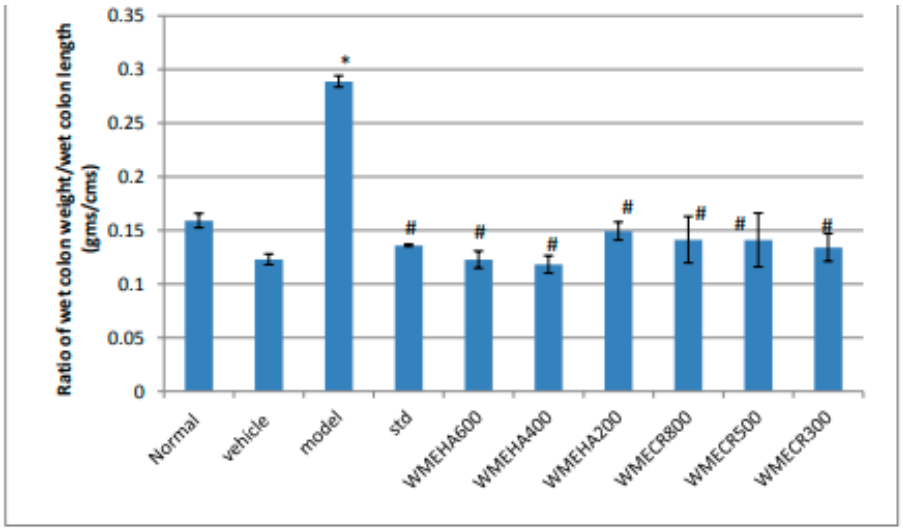

Figure 2: Effect of WMEHA and WMECR on colon wt/l in DNBS induced inflammatory bowel disease in rats

Effects of WMEHA and WMECR on colon mucosal damage index (CMDI) in DNBS induced inflammatory bowel disease in rats:

The effects of WMEHA and WMECR on colon mucosal damage index (CMDI) in DNBS-induced inflammatory bowel disease in rats were examined. Intrarectal injection of DNBS (120 mg/kg in 50% ethanol) induced mucosal disruption, linear and deep ulcers, bleeding, and submucosal edema, resulting in significant mucosal damage observed upon macroscopic examination of the colon section closest to the injection site.

Compared to normal control animals, the CMDI scores of the model control group (3.667±0.333; $P<0.05$) were significantly higher after intracolonic administration of DNBS. Conversely, the CMDI scores of the normal control group were notably lower (0.0±0.0; $P<0.001$). Animals subjected to vehicle control did not exhibit a significant difference in CMDI scores compared to healthy controls. Treatment with 5-ASA (100 mg/kg, p.o. for 18 days) led to a substantial reduction in CMDI score (0.0±0.0; $P<0.001$) compared to DNBS control animals. Moreover, animals treated with WMEHA (200, 400, and 600 mg/kg, p.o. for 18 days) and WMECR (500 and 800 mg/kg, p.o. for 18 days) showed significantly reduced CMDI scores ranging from 0.0±0.0 to 1.0±1.0 compared to DNBS control animals.

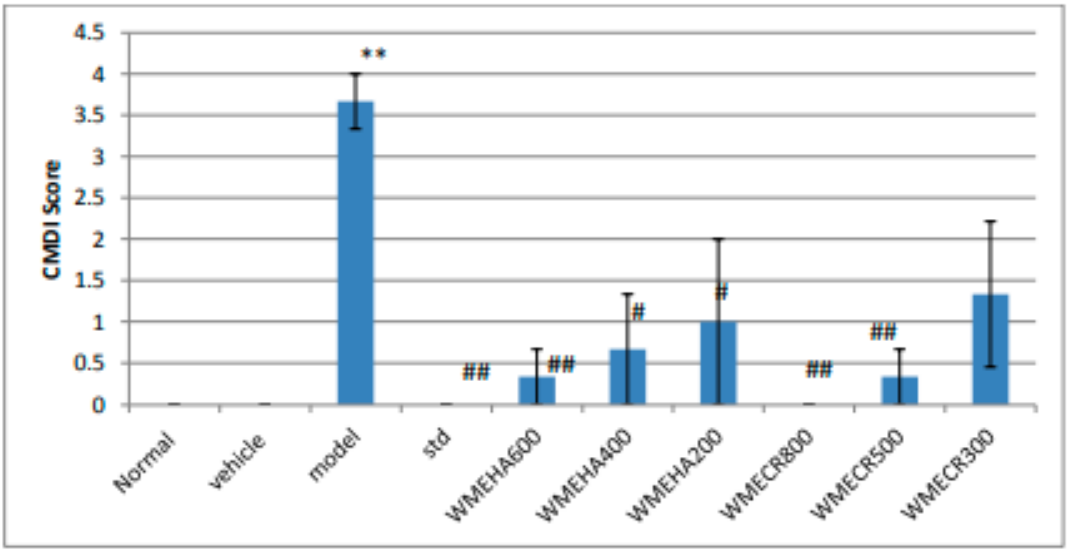

Figure 1: Effect of WMEHA and WMECR on CMDI in DNBS induced inflammatory bowel disease in rats

3.4 Impact of Solvent Extracts on Stool Consistency in Rat IBD

Holarrhena antidysenterica and Cyperus rotundus were subjected to sequential extraction using different solvents including petroleum ether, hexane, chloroform, acetone, and hydromethanol. The impact of these extracts on stool consistency in rats with DNBS-induced inflammatory bowel disease was evaluated.

The average stool consistency score significantly increased in the model control group (1.67±0.33; $P<0.001$) compared to normal control animals (0.0±0.0; $P<0.05$) after DNBS administration. However, treatment with MEHA (600 mg/kg, p.o. for 18 days) and HECR (800mg/kg, p.o. for 18 days), CHCR (800mg/kg, p.o. for 18 days), ACCR (800mg/kg, p.o. for 18 days), and MECR (800mg/kg, p.o. for 18 days) significantly decreased the stool consistency score, ranging from 0.33±0.33 to 0.0±0.0, compared to DNBS control animals (Figure 4.4). Remarkably, a 100% improvement was observed in rats treated with HECR (800mg/kg), CHCR (800mg/kg), ACCR (800mg/kg), and MECR (800mg/kg).

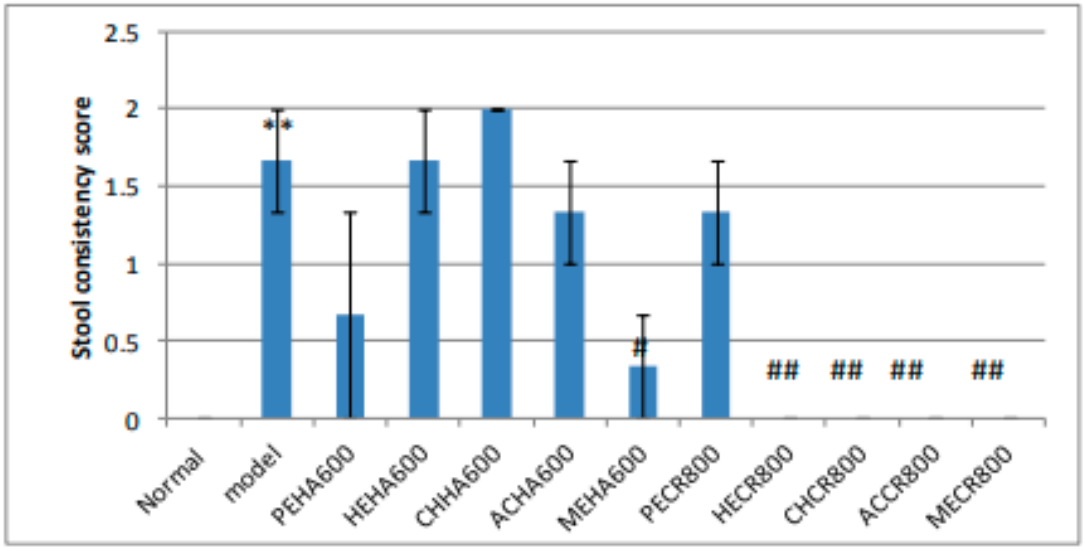

Figure 5: Effect of various solvents extracts of Holarrhena antidysenterica and Cyperus rotundus on stool consistency in DNBS induced inflammatory bowel disease in rats.

Effects of Extracts on Colon Weight/Length Ratio

The wet colon weight/length ratio significantly increased in the model control group following intracolonic administration of DNBS compared to normal control animals (0.288±0.005 gm/cm vs. 0.186±0.003 gm/cm; P<0.05). Treatment with PEHA, CHHA, MEHA, HECR, CHCR, ACCR, and MECR significantly decreased the wet colon weight/length ratio compared to DNBS control animals (0.198±0.007 gm/cm, 0.193±0.005 gm/cm, 0.1117±0.002 gm/cm, 0.199±0.046 gm/cm, 0.12±0.01 gm/cm, 0.158±0.027 gm/cm, 0.147±0.018 gm/cm, respectively; P<0.05 or P<0.001). The statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Dunnett's Post hoc test, with significance levels set at P<0.05 and P<0.001.

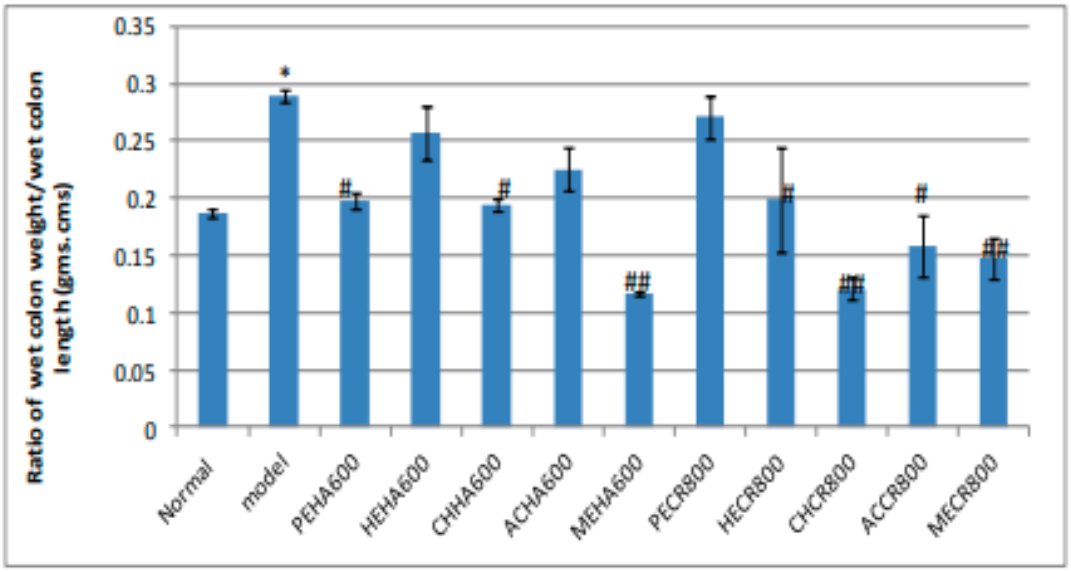

Figure 6: Effect of various solvents extracts of Holarrhena antidysenterica and Cyperus rotundus on colon wt/l in DNBS induced inflammatory bowel disease in rats

3.5 Effect of MEHA and CHCR on histopathology of organs

Pathological investigations of male and female rats treated with MEHA150, MEHA450, MEHA1350, CHCR200, CHCR600, and CHCR1800 revealed no lesions or pathological alterations in the heart, liver, lungs, or kidneys. Degeneration, fibrosis, and necrosis were not seen in microscopic examinations of tissues from treated rats. Liver slices from all treatment groups revealed no fatty infiltration and a typical cellular architecture characterised by discrete hepatocytes, sinusoidal gaps, and a central vein. Lung tissue sections treated with these methods showed no abnormalities

in their vascular networks, alveoli, or interstitial spaces. There were no signs of vascular engorgement or bleeding, alveolar dilatation, inflammatory cell infiltration, or interstitial fibrosis. Kidney sections from all treatment groups showed healthy parenchyma, free of bleeding and necrosis, similar to those from the control group. Sections of hearts from all the groups showed normal, striated muscle fibers. Since the histopathological results were the same in untreated and treated rats, this suggests that there was no toxicological impact from administering any of the three doses of either extract.

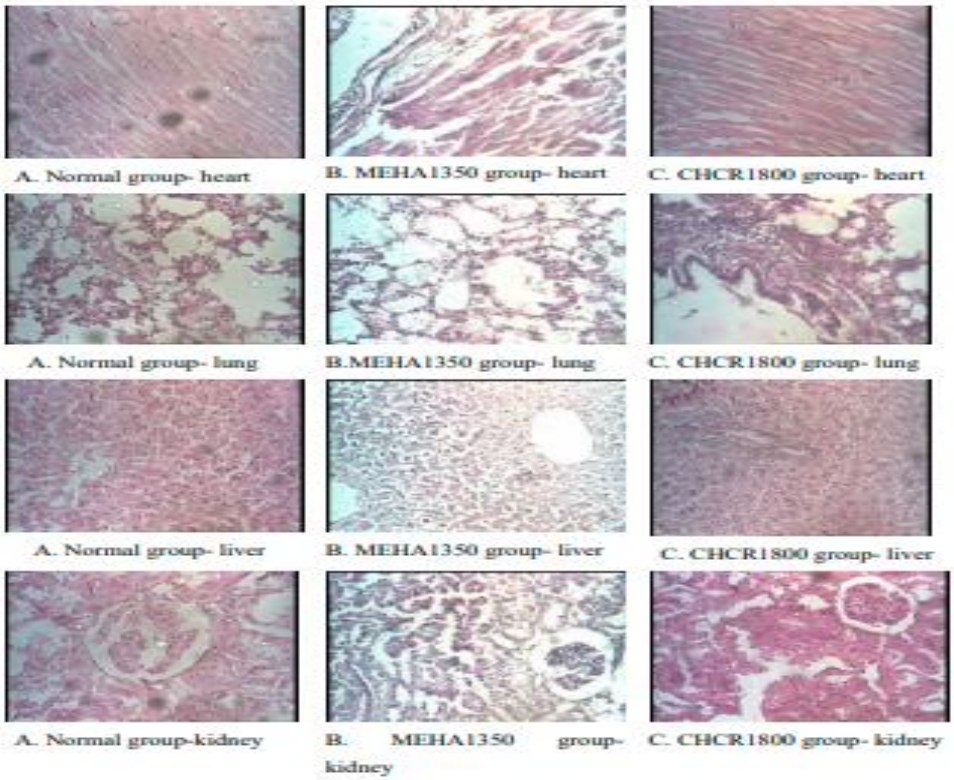

Figure 7: Light micrographs of tissue sections from rats of the different experimental groups (H & E stain, 100X) ((A) Normal group- heart (B) MEHA1350 group- heart (C) CHCR1800 group- heart (A) Normal group- lug (B) MEHA1350 group- lung (C) CHCR1800 group- lung (A) Normal group- liver (B) MEHA1350 group- liver (C) CHCR1800 group- liver (A) Normal group – kidney (B) MEHA1350 group- kidney (C) CHCR1800 group- kidney

Table 2: Haematological parameters of MEHA treated rats in sub acute toxicity

Haematological parameters	**MALE**				**FEMALE**			
(Normal Values)	**Contro l**	**MEHA(mg/kg)**			**Contro l**	**MEHA(mg/kg)**		
		150	450	1350		150	450	1350
RBC(x106/mm3) (7-10)	7.492+ 0.407	7.866+ 0.169	7.552+ 0.178	7.232+ 0.214	7.712+ 0.271	7.32+ 0.287	6.96+ 0.276	7.13+ 0.257
Hb (g/dl) (11-18)	12.9+ 0.693	13.86 0.384	14.56+ 0.957	14.23+ 0.274	14.98+ 2.973	13.02 0.662	13.22+ 0.654	12.85+ 0.593
MCV(fl) (36 -58)	49.76+ 1.388	49.46+ 0.915	50.62+ 1.62	51.01+ 0.892	50.84+ 1.399	48.18+ 0.821	41.96+ 9.386	43.59+ 7.316
MCH (pg) (17.1-20.4)	17.24+ 0.623	17.6+ 0.313	17.9+ 0.524	17.5+ 0.374	17.7+ 0.317	17.48+ 0.269	18.24+ 0.344	18.69+ 0.195
MCHC (g/dl) (32.9-37.5)	34.9+ 0.366	34.54+ 0.337	34.44+ 0.204	36.42+ 0.295	34.9+ 0.557	34.64+ 0.663	34.94+ 1.095	34.27+ 0.583
WBC(x103/mm3) (6-17)	9.84+ 1.598	11.06+ 1.899	10.86+ 2.096	11.94+ 1.874	8.5+ 1.003	9.62+ 0.678	8.64+ 0.571	8.96+ 0.426
Lymphocytes (%) (65-85)	64.06+ 7.608	73.64+ 2.375	68.96+ 1.666	68.58+ 2.016	69.87+ 3.066	71.78+ 1.55	69.02+ 2.031	70.39+ 1.865
Monocytes(%) (0-5)	4.02+ 1.873	2.78+ 1.119	4.56+ 0.601	3.48+ 0.512	4.82+ 0.974	2.98+ 0.824	4.64+ 0.571	3.79+ 0.742

Granulocytes (%) (9-34)	19.42+ 4.751	13.58+ 1.315	16.48+ 1.225	14.63+ 1.752	13.22+ 1.205	14.36+ 0.823	14.94+ 0.733	16.01+ 0.672
PLT(x103/mm3)(500-1300)	573.6+70.77	634.2+22.24	654.2+24.44	657.1+22.39	581.6+69.85	659.6+21.64	674.4+26.13	668.3+22.69

Table 3: Haematological parameters of CHCR treated rats in subacute toxicity

Haematological parameters	MALE				FEMALE			
(Normal Values)	Contro l	CHCR(mg/kg)			Contro l	CHCR(mg/kg)		
		200	600	1800		200	600	1800
RBC(x106/mm3) (7-10)	7.492+ 0.407	6.58+ 0.768	7.176+ 0.554	7.576+ 0.214	7.712+ 0.271	6.84+ 0.278	7.272+ 0.537	7.579+ 0.316
Hb (g/dl) (11-18)	12.9+ 0.693	11.68+ 1.667	12.92+ 1.074	11.57+ 1.021	14.98+ 2.973	11.26+ 0.881	12.24+ 0.721	13.45+ 0.593
MCV(fl) (36 -58)	49.76+ 1.388	52.02+ 0.521	52.36+ 0.898	50.47+ 0.476	50.84+ 1.399	52.02+ 0.836	52.8+ 0.739	51.98+ 0.628
MCH (pg) (17.1-20.4)	17.24+ 0.623	17.34+ 0.742	17.96+ 0.341	16.64+ 0.496	17.7+ 0.317	17.12+ 0.422	17.28+ 0.318	16.57+ 0.529
MCHC (g/dl) (32.9-37.5)	34.9+ 0.366	33.62+ 1.288	34.24+ 0.462	33.57+ 0.396	34.9+ 0.557	32.42+ 1.751	33.74+ 0.578	34.13+ 1.103
WBC(x103/mm3) (6-17)	9.84+ 1.598	7.42+ 1.661	7.2+ 1.431	6.98+ 1.528	8.5+ 1.003	6.86+ 0.75	7.06+ 0.88	7.97+ 0.59
Lymphocytes (%) (65-85)	64.06+ 7.608	69.96+ 7.169	76.42+ 1.363	74.21+ 2.498	69.87+ 3.066	66.16+ 2.927	73.3+ 1.641	70.26+ 1.267
Monocytes(%)	4.02+	4.71+	1.76+	2.47+	4.82+	3.9+	1.52+	2.27+
(0-5)	1.873	2.5	0.417	0.729	0.974	1.293	0.617	0.391
Granulocytes (%) (9-34)	19.42+4.751	14.34+4.673	11.82+1.22	14.65+2.37	13.22+1.205	13.08+1.327	12.26+.319	12.68+1.402
PLT(x103/mm3) (500-1300)	573.6+70.77	561.2+68.94	548.4+20.4	544.3+36.8	581.6+69.85	569.4+77.59	553.6+22.72	561.7+20.31

3.6 Main Efficacy Studies

In this study, the effects of MEHA (450 and 600 mg/kg) and CHCR (600 and 800 mg/kg) on various parameters of DNBS-induced inflammatory bowel disease (IBD) in rats were investigated. When compared to healthy controls, the physical, macroscopic, biochemical, and histological characteristics remained largely unchanged in rats treated with the vehicle, 50% ethanol. This indicated that DNBS, and not the vehicle, was responsible for inducing IBD. Moreover, treatment with CMC suspension, an inert agent used to suspend the extracts, did not show significant efficacy in curing IBD, as evidenced by the lack of changes in physical, macroscopic, biochemical, and histological parameters compared to the model group.

The physical parameters of DNBS-induced IBD were established through daily monitoring of weight change, food intake, water intake, stool consistency, wet colon weight, and wet colon length measurements at the end of the study. DNBS model control animals exhibited significant weight loss, reduced water and food intake, altered stool consistency, and decreased wet colon length compared to normal control animals. However, treatment with 5-ASA, MEHA, and CHCR significantly attenuated these effects, indicating their potential in mitigating the symptoms of IBD.

Macroscopic examination revealed significant mucosal damage in the DNBS-induced model control group, characterized by mucosal rupture, ulcers, bleeding, and edema. Treatment with 5-ASA, MEHA, and CHCR resulted in normal colon appearance, indicating successful mitigation of DNBS-induced damage.

Biochemical analysis showed that DNBS administration led to increased myeloperoxidase activity and malondialdehyde levels, indicative of neutrophil infiltration and lipid peroxidation, respectively. However, treatment with 5-ASA, MEHA, and CHCR significantly reduced these parameters, suggesting inhibition of inflammation and oxidative stress. Additionally, these treatments prevented the increase in nitric oxide levels and restored superoxide dismutase activity in colonic tissue, further highlighting their anti-inflammatory and antioxidant effects.

Serum cortisol levels were significantly elevated in DNBS-induced model control animals, indicating stress response. Treatment with 5-ASA, MEHA, and CHCR effectively decreased serum cortisol levels, suggesting a reduction in stress associated with IBD.

Histopathological examination revealed extensive tissue damage, inflammation, and leukocyte infiltration in the DNBS-induced model control group. In contrast, treatment with MEHA (450 and 600 mg/kg) and CHCR (800 mg/kg) showed minimal mucosal damage, reduced inflammation, and preserved mucosal integrity, indicating their protective effects against DNBS-induced colitis.

Overall, treatment with MEHA and CHCR demonstrated significant efficacy in ameliorating the symptoms and pathological changes associated with DNBS-induced IBD in rats, highlighting their potential as therapeutic agents for the management of IBD.

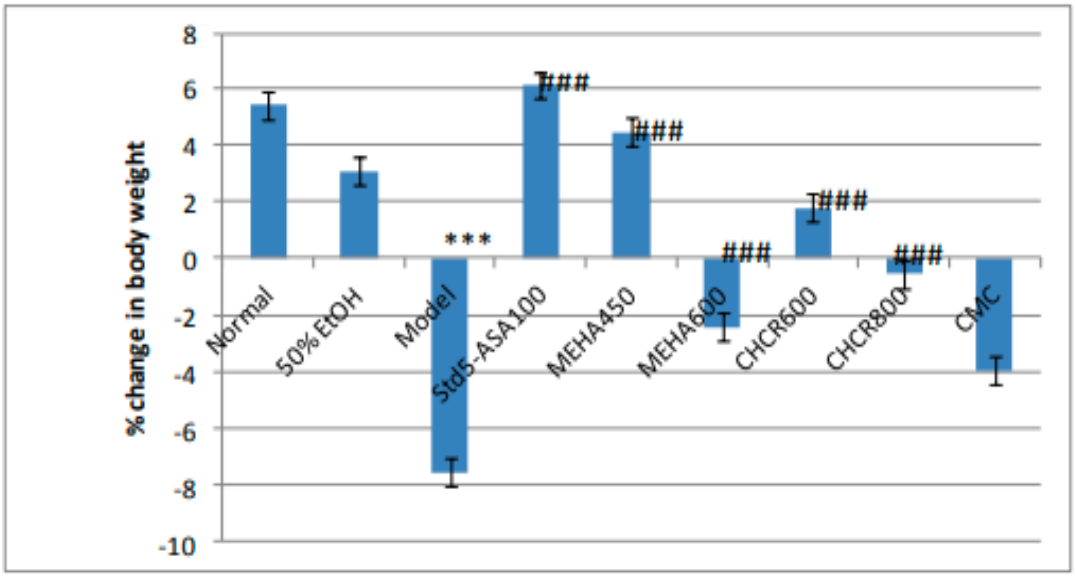

Figure 8: Effect of MEHA and CHCR on % change in body weight DNBS induced inflammatory bowel disease in rats.

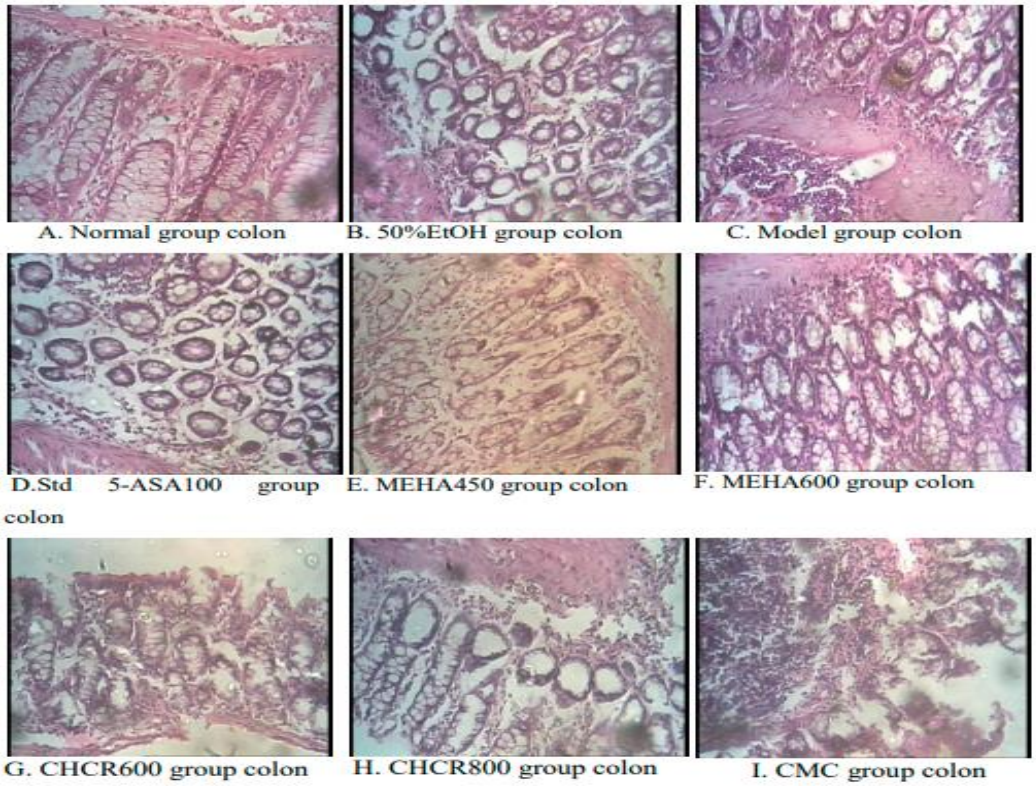

Figure 9: Histological appearance of rat colonic tissues.

4. Conclusion

In this research article, the efficacy of Holarrhena antidysenterica (MEHA) and Cyperus rotundus (CHCR) extracts in the treatment of colitis was investigated. The study demonstrated promising therapeutic potential for both MEHA and CHCR extracts, as evidenced by improvements in various parameters such as colon macroscopic damage, biochemical markers, and histopathological findings.

MEHA and CHCR extracts showed significant anti-inflammatory effects and ameliorated colitis symptoms in the experimental model. These effects were accompanied by modulation of oxidative stress markers and preservation of colon histology. Notably, MEHA and CHCR extracts exhibited comparable efficacy to standard medicine (5-ASA), indicating their potential as alternative or adjunctive therapies for colitis management.

The findings of this study support further exploration of MEHA and CHCR extracts as potential therapeutic agents for inflammatory bowel diseases. Future research should focus on elucidating the underlying mechanisms of action and conducting clinical trials to validate their efficacy and safety in human subjects. Overall, this study contributes valuable insights into the pharmacological properties of MEHA and CHCR extracts and their potential utility in the management of colitis.

Author statement

All individuals who fit the criteria for authorship have been credited, and they attest to having made substantial contributions to the conception, research, analysis, writing, and review of the text.

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Conflict of interests

Authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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