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Medicinal Herb Extracts In Chronic Colitis: Amelioration Of Inflammation And Restoration Of Intestinal Barrier Integrity

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Abstract
Medicinal herbs, known for their bioactive phytochemicals such as polyphenols and flavonoids, exhibit anti-inflammatory, antioxidant, and gut-protective properties, offering a potential therapeutic method for restoring intestinal homeostasis. Evaluating the impact of medicinal plant extracts on intestinal barrier restoration and inflammation reduction in a chronic colitis model was the main objective of this research. Experimental data were obtained using a murine model of colitis made by chemical irritants, with groups receiving either a standard diet or a diet supplemented with a defined dose of herbal extracts. Inflammatory markers, oxidative stress parameters, and intestinal barrier integrity indicators were systematically measured. Analyses involved quantitative polymerase chain reaction, enzyme activity assays, and histopathological evaluations, alongside gut microbiota profiling to assess the modulatory effects on microbial communities. Statistical evaluation was conducted using ANOVA and post-hoc comparisons in IBM SPSS version 28.0 to determine significant differences among experimental groups (EG). The results demonstrated that herbal extract supplementation significantly decreased pro-inflammatory cytokines and oxidative stress levels while enhancing antioxidant enzyme activities and expression of tight junction proteins, indicating improved barrier integrity. Herbal extract supplementation significantly reduced TNF-α from 120.4 ± 5.6 to 78.3 ± 4.2 pg/mg and IL-6 from 98.7 ± 4.9 to 62.5 ± 3.7 pg/mg at day 14, while restoring tight junction protein expression. Positive changes in the makeup of intestinal microbes were also noted. According to these results, extracts from medicinal herbs are used to treat chronic colitis by efficiently reducing colonic oedema and enhancing the healing function of the gut barrier.

Keywords: *Chronic Colitis, Medicinal Herb Extracts, Inflammation, Intestinal Barrier Integrity, Oxidative Stress, Gut Microbiota, Tight Junction Proteins*

1. INTRODUCTION

Inflammatory bowel disease (IBD) is a major public health issue that represents a chronic, relapsing gastrointestinal condition, with an increasing incidence globally [1]. Colitis can develop due to various risk factors, including environmental, genetic, and dietary risk factors, and colitis symptoms are often severe and are associated with an inflammatory response characterized by infiltration of immune cells into the tissue [2]. Segmental infection is a hallmark of Crohn's disease (CD), which also permits the creation of granulomas, facilitating its recurrent and chronic disposition [3]. As stated by the World Health Organization (WHO), more than 80% of the world's population depends on traditional medicine for health. Herbal products, used for thousands of years, provide reduced side effects, improved availability, and affordability [4]. Traditional medicine has many bioactive ingredients that may aid in the treatment of IBD, even though its effects and mechanisms are not entirely understood. Many herbal components have been shown to reduce colitis through multiple pathways, including partially preventing inflammation by regulating inflammasome activation [5].

1.1 Research aim

Research investigates whether the anti-inflammatory, antioxidant, and gut-protective properties of medicinal herb extracts can help treat chronic colitis. Its particular objectives were to determine which herbal ingredients were best for repairing the integrity of the intestinal block, assess their effects on pro-inflammatory cytokines and antioxidant enzyme activities, and find therapeutic pathways that aid in the treatment of chronic colitis.

1.2 Research framework

Herbal extracts as a treatment for chronic colitis are introduced in Section 1. Anti-inflammatory, antioxidant, and gut-protective properties are reviewed in Section 2. ANOVA with post-hoc testing, sample collection, treatment groups, and 36 mice are covered in Section 3. Section 4 displays better microbiota, decreased oxidative stress, inflammation, and barrier integrity. Future research and therapeutic potential are highlighted in Section 5.

2. RELATED WORKS

Performed investigations using animal models of ulcerative colitis (UC) to test natural substances and herbal decoctions [6]. Results indicated that these treatments enhanced intestinal barrier function, reduced colonic inflammation, and altered gut microbiota via pathways. Preclinical models with little clinical validation, however, provide the majority of the evidence. Preclinical research studies looked into the effect of Traditional Chinese Medicine (TCM) on gut microbiota in ulcerative colitis with the purpose of restoring microbial homeostasis and protecting the intestinal barrier [7]. TCM led to an increase in beneficial microbiota while decreasing pathogenic microbiota, and it enhanced tight junction integrity along with promoting mucosal regeneration. Most of the evidence supporting TCM for diet-induced alterations in gut microbiota was preclinical, and human studies with modern methodologies were warranted to understand the effects and mechanisms. Ginger polysaccharides (GP) have been evaluated for their potential to cure UC via altering intestinal inflammation, barrier function, and gut microbiota [8]. Results indicate that GP decreases the production of various pro-inflammatory cytokines, repairs intestinal barrier proteins, and modifies gut microbiota to ameliorate UC symptoms. However, results were limited to preclinical models, and safety and efficacy in humans must be assessed in other studies. The way Chinese Herbal Medicine Formulas (CHMF) are used to treat IBD was investigated [9]. The findings showed that CHMF can lessen oxidative damage, inflammation, and intestinal barrier function. More research was required to elucidate its therapeutic mechanisms and enhance efficacy. Turmeric-derived nanovesicles (TNVs) were designed as a treatment for UC [10]. The findings demonstrated that TNVs had a significant anti-colitic effect by reducing inflammation and restoring the function of the intestinal barrier. To confirm safety, efficacy, and mechanisms of action, more human research was needed.

3. METHODOLO#GY

A control group (CG), an experimental group (EG), and a group treated with the herbal extract were the two groups to which 36 male mice suffering from chronic colitis were randomly allocated. On days 0, 14, and 28, the research gathered fecal samples, colon tissues, and clinical symptoms. To evaluate the impact of herbal supplementation on inflammation, barrier integrity, and microbial composition, the research used ANOVA with post hoc tests. The overall flow diagram is shown in Figure 1.

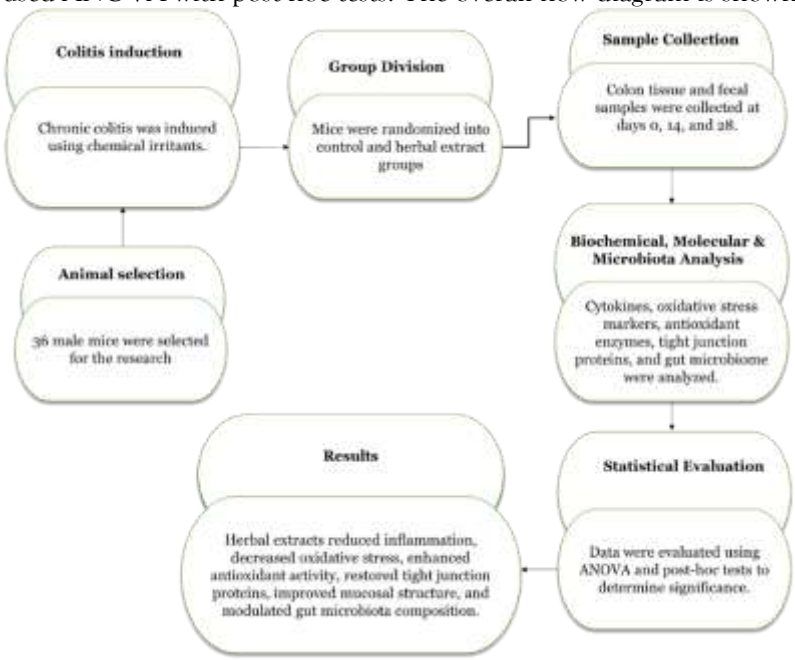

Figure 1. Overall methodology flow diagram

3.2 Data collection

36 male mice, ages 8 to 10 weeks, were used in the research; each EG and CG contain 18 male mice's. Colitis was induced in all animals to achieve n = 6 biological replicates per group per time point for tissue analysis. Six mice each group had baseline (pre-treatment) samples taken on day 0. The remaining six mice in each group were put to death at the endpoint (day 28), while six more mice were slaughtered at the halfway point (day 14). Both the experimental and control conditions had a constant sample size throughout all time points because to this design. Every day, clinical measurements such as body weight,

stool score, and bleeding were noted for each of the 36 animals. Stool samples were collected from each mouse at days 0, 7, 14, 21, and 28 (longitudinal microbiota profiling; 5 timepoints × 18 samples/group). Following collection, the colon tissue was separated into three sections: one was preserved in RNA and then cooled to -80°C for Quantitative Polymerase Chain Reaction (qPCR) (n = 6), one was snap-frozen for biochemical tests (Malondialdehyde (MDA) and Superoxide Dismutase (SOD); n = 6), and one was fixed in 10% formalin for histology (H&E; n = 6). Technical triplicates were used for all molecular experiments, and sequencing employed a single sample per mouse (no pooling).

3.3 Group Separation and Treatment Administration

After inducing colitis in mice, they were randomly divided into two groups receiving either a standard diet or one supplemented with therapeutic herb extracts. The treatment continued for several weeks to replicate chronic disease conditions. Randomization lessened selection bias, and all groups were maintained in identical housing conditions. Tissue samples were taken at scheduled time points for assessment of tight junction proteins, oxidative stress, inflammatory markers, and gut microbial composition; allowing for a direct comparison between models of colitis, treated and untreated.**3.3.1**

3.3.1 Control Group

Mice with colitis were kept on a regular diet without any supplements. Obtained the identical monitoring and sampling experiences like the EG. The baseline for assessing oxidative stress, inflammation, barrier integrity, and microbial alterations was tissue and fecal samples from control animals.

3.3.2 Experimental Group

The number of extracts of medicinal herbs that were added to the daily meal of mice with colitis was determined and administered to the mice throughout the investigation. The same clinical monitoring and sample collection procedures were done to the control group. This group was used to test the positive effect of herbal extracts on the tight junction protein expression, oxidative stress regulation, gut microbiota composition and reduction of inflammation.

3.4 Statistical analysis

The experiment's data was analyzed utilizing the IBM Statistical Package of the Social Sciences (SPSS) version 28.0, which was employed to determine significant changes in a group. Post-hoc group comparisons for significant time points was conducted for both the control and EG groups. Data are reported as mean SD ($P < 0.05$). This method of analysis established the effects of the herbal extract on tight junction proteins, oxidative stress, gut microbiota, histopathology scores and antioxidant enzyme activities.

3.5.1 Analysis of Variance (ANOVA)

ANOVA was utilized to evaluate the effect of medicinal herb extract supplementation on colitis-related biomarkers. The analysis consisted of a Repeated Measures ANOVA, for identifying differences in pro-inflammatory cytokines, oxidative stress markers, antioxidant enzyme activity, and intestinal barrier. This analysis enabled evaluation of the consistency of improvements across metrics, specifically across pathways, by partitioning total variability to between group variability and within group variability to determine differences in significance levels. The mathematical formulation of ANOVA is expressed in equation (1).

$$F = \frac{MS_{between}}{MS_{within}} \quad (1)$$

Where $MS_{between}$ represents the variability due to treatment (standard diet vs. herb-supplemented diet) and MS_{within} represents variability within groups. A larger F-value indicates stronger evidence of treatment effectiveness across the measured biomarkers.

3.5.2 Post-hoc test

The treatment groups that showed significant differences in pro-inflammatory cytokines, oxidative stress indicators, antioxidant enzyme activities, and intestinal barrier integrity were identified using post-hoc analysis. For every pairwise comparison between the control and herbal extract supplementation groups, Tukey's HSD test was used. Each comparison's critical difference was determined using equation (2).

$$HSD_{jk} = q_{\alpha,l.eg}\sqrt{\frac{MSE}{2}\left(\frac{1}{p_j} + \frac{1}{p_k}\right)} \quad (2)$$

Where HSD_{jk} is the Honestly Significant Difference between group means j and k, then $q_{\alpha,}$ l. e.g., is the critical value from the studentized range distribution at significance level α with l groups and eg degrees of freedom. MSE is the pooled mean square error, and p_j and p_k are the sample sizes of the groups. A mean difference exceeding HSD indicates a statistically significant effect of herbal extracts on the respective biological indicator, highlighting the most responsive therapeutic pathways.

4. RESULT

Supplementing mice with herbal extracts dramatically decreased oxidative stress and pro-inflammatory cytokines in mice with colitis. Significant improvements were observed in tight junction protein expression and antioxidant enzyme activity. In histology, mucosal structure was restored, and the gut microbiota changed to include more advantageous species. Generally, medicinal herbs effectively decreased inflammation and strengthened the intestinal barrier.

4.1. Inflammatory Markers in Chronic Colitis

To evaluate the anti-inflammatory impact of medicinal plant extracts, measurements were made of the colonic inflammatory cytokines Interleukin (IL)-6, Tumor Necrosis Factor (TNF)-α, and IL-1β. Significant differences between the groups were found by an ANOVA at days 14 and 28 ($p < 0.001$). Post-hoc comparisons showed that the EG had significantly lower levels of all cytokines compared to the control group ($p < 0.01$). Baseline (day 0) cytokine levels were similar across groups, confirming effective randomization. Histopathological scoring of colon tissue corroborated molecular findings, showing reduced immune cell infiltration and improved mucosal structure in the EG. Table 1 results demonstrate that herbal extracts effectively reduce colonic inflammation in chronic colitis. Figure 2 shows the changes in pro-inflammatory species.

Table 1: Inflammatory Markers (pg/mg tissue, mean ± SD)

Timepoint	Group	TNF-α	IL-6	IL-1β
Day 0	Control (n=6)	45.2 ± 3.1	38.5 ± 2.8	30.7 ± 2.4
	Experimental (n=6)	44.8 ± 3.2	38.2 ± 3.0	30.5 ± 2.5
Day 14	Control	120.4 ± 5.6	98.7 ± 4.9	85.6 ± 5.1
	Experimental	78.3 ± 4.2 **	62.5 ± 3.7 **	55.2 ± 3.5 **
Day 28	Control	135.7 ± 6.1	112.3 ± 5.4	95.8 ± 5.7
	Experimental	70.1 ± 3.8 **	58.9 ± 3.2 **	50.7 ± 3.0 **

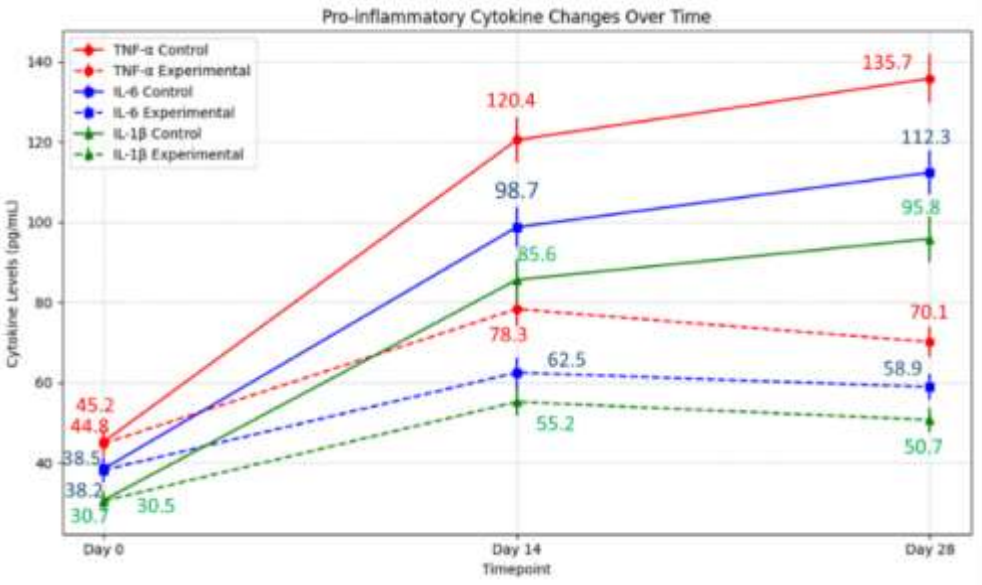

Figure 2: Representation of pro-inflammatory cytokine changes

4.2. Oxidative Stress and Antioxidant Activity

Oxidative stress was assessed by measuring Malondialdehyde (MDA) levels and antioxidant enzyme activities. ANOVA showed significant differences among groups at the mid-point and end-point ($p < 0.001$). Post-hoc tests indicated that herbal extract supplementation significantly reduced MDA levels while enhancing Superoxide Dismutase (SOD) and catalase activities ($p < 0.01$). Baseline values were comparable across groups. These findings indicate that herbal extracts protect against oxidative damage while boosting endogenous antioxidant defenses, contributing to improved colonic health. Table 2 shows the findings. Figure 3 shows the comparison of both groups with oxidative stress.

Table 2: Oxidative Stress and Antioxidant Enzymes

Timepoint	Group	MDA (nmol/mg)	SOD (U/mg)	Catalase (U/mg)
Day 0	Control (n=6)	2.5 ± 0.2	15.4 ± 1.1	18.2 ± 1.3

	Experimental (n=6)	2.4 ± 0.2	15.6 ± 1.2	18.1 ± 1.2
Day 14	Control	6.8 ± 0.4	10.2 ± 0.9	12.1 ± 0.8
	Experimental	3.9 ± 0.3 **	18.5 ± 1.0 **	20.4 ± 1.2 **
Day 28	Control	7.5 ± 0.5	9.8 ± 0.8	11.7 ± 0.7
	Experimental	3.5 ± 0.3 **	19.2 ± 1.1 **	21.0 ± 1.3 **

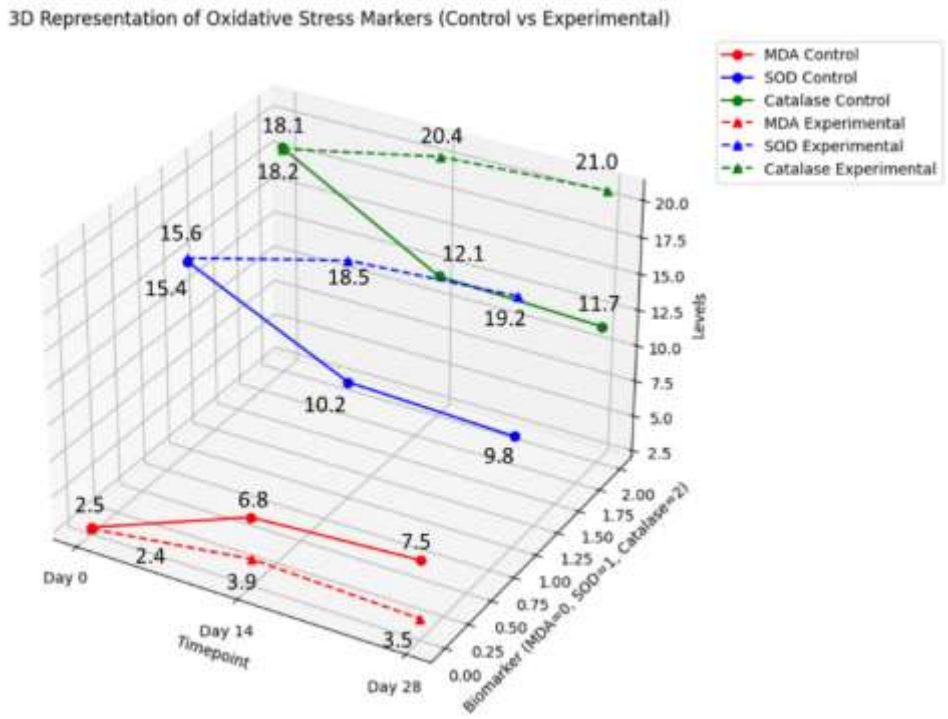

Figure 3: Representation of Oxidative stress

4.3. Intestinal Barrier Integrity and Microbiota

The function of the intestinal barrier was assessed by measuring tight junction proteins such as claudin-1, occluding, and Zonula Occludens (ZO)-1. ANOVA showed that the groups differed in a highly significant way ($p < 0.001$). Post-hoc analyses revealed that supplementing with herbal extracts significantly increased tight junction expression on days 14 and 28 ($p < 0.01$). Histological examinations verified that the experimental group's epithelial architecture had improved and that their mucosal damage metrics had decreased. Gut microbiota analysis revealed that treated animals had increased beneficial bacterial taxa and reduced pro-inflammatory species. Data suggest that the fermentation extracts of the medicinal herb can improve barrier integrity and alter gut microbiota, leading to protective effects in chronic colitis, as represented by Table 3. Figure 4 shows the outcome of the tight junction protein.

Table 3: Tight Junction Protein Expression

Timepoint	**Group**	**Occludin**	**Claudin-1**	**ZO-1**
Day 0	Control (n=6)	1.00 ± 0.05	1.02 ± 0.04	1.01 ± 0.05
	Experimental (n=6)	1.01 ± 0.04	1.03 ± 0.05	1.02 ± 0.05
Day 14	Control	0.55 ± 0.03	0.60 ± 0.04	0.58 ± 0.03
	Experimental	0.92 ± 0.04 **	0.95 ± 0.03 **	0.94 ± 0.04 **
Day 28	Control	0.50 ± 0.04	0.55 ± 0.03	0.53 ± 0.03
	Experimental	0.96 ± 0.03 **	0.98 ± 0.04 **	0.97 ± 0.03 **

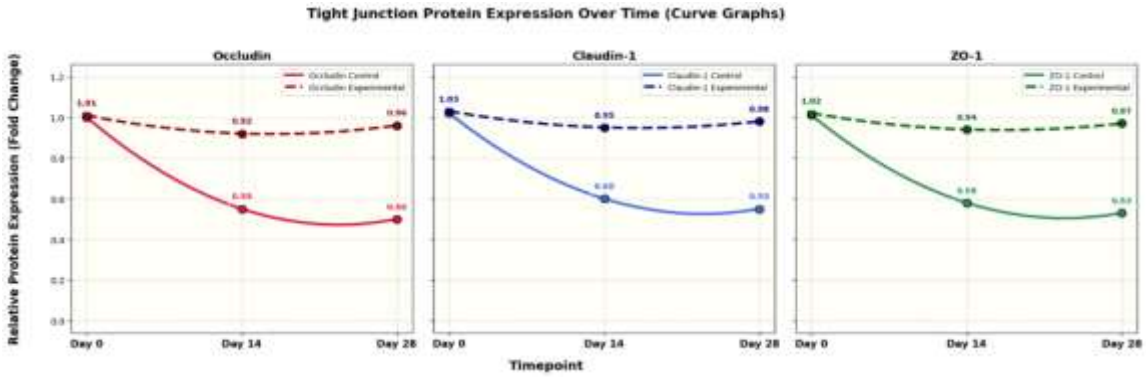

Figure 4: Representation of the Tight junction protein

5. DISCUSSION

Preclinical research indicates that herbal decoctions, ginger polysaccharides, and turmeric-derived nanovesicles restore intestinal barrier function, reduce colonic inflammation, and modulate gut microbiota [6–8]. Formulations based on Traditional Chinese Medicine also show improvements in microbial composition, immune regulation, and mucosal repair. Overall, much of this evidence comes from animal model studies, and the multi-component nature of the formulations complicates the understanding of mechanisms of action [7-9]. The mentioned limitations may potentially be minimized through systematic studies using a controlled experimental design with a defined dose, longitudinal sampling, and a comprehensive assay of molecular, histological, and microbiota analysis. Statistical methods such as ANOVA and post-hoc testing would also be more suitable in taking into account the issues of efficacy and possible mechanisms of action that can be applied to translating the herbal interventions to subsequent clinical use.

6. CONCLUSION

The research was to assess the effects of extracts of medicinal herbs on the colon by assessing the inflammatory markers, oxidative stress, antioxidant activities, and tight junction protein expression and gut microbiota in 36 heterologous mice with chronic colitis. The greatest reductions in inflammatory markers were observed for TNF-α (Control M = 135.7 ± 6.1, Experimental M = 70.1 ± 3.8, $p < 0.001$) and IL-6 (Control M = 112.3 ± 5.4, Experimental M = 58.9 ± 3.2, $p < 0.001$), respectively. The experimental herb extracts reduced MDA levels from 7.5 ± 0.5 to 3.5 ± 0.3 nmol/mg ($\Delta = 4.0$, $p < 0.001$). Tight junction proteins were statistically different using an ANOVA among groups ($F = 12.34$, $p < 0.001$). The reliability of the preclinical models, the sample size, and the brief duration of the intervention were recognized to have caveats on the reliability. Further research will require the establishment of herb intervention effectiveness and clinical usability in the environment of human trials, multiple model validation, longitudinal studies, and mechanistic studies.

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