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COMPARATIVE ASSESSMENT OF APIGENIN MONOTHERAPY AND ITS COMBINATION WITH VITAMIN B2 ON CLINICAL AND HEMATOLOGICAL OUTCOMES IN ACETIC ACID-INDUCED ACUTE COLITIS IN RATS

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Keywords
Apigenin; riboflavin; vitamin B2; acute colitis; acetic acid; Disease Activity Index; oxidative stress; inflammation; hematological parameters; Wistar rats.

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Abstract

Background: *Acute colitis is characterized by intestinal mucosal injury, oxidative stress, and excessive inflammatory responses. Natural bioactive compounds with antioxidant and anti-inflammatory properties may provide protective effects against colonic inflammation. Apigenin, a dietary flavonoid, and riboflavin (vitamin B_2), an essential cofactor involved in cellular redox metabolism, have individually demonstrated potential against oxidative and inflammatory processes; however, their combined effects in chemically induced acute colitis require further investigation.*

Objective: *The present study evaluated the protective effects of apigenin and vitamin B2, administered individually and in combination, against acetic acid-induced acute colitis in rats, with particular emphasis on disease activity, behavioral and physiological changes, and hematological alterations.*

Methods: *Thirty healthy adult albino Wistar rats weighing 200–300 g were randomly allocated into five groups (n = 6): normal control, positive control, standard treatment, apigenin-treated, and combination-treated groups. Acute colitis was induced using 2% glacial acetic acid. Apigenin (50 mg/kg body weight), vitamin B2 (100 mg/kg body weight), and omeprazole (20 mg/kg body weight) were administered orally according to the experimental treatment schedule for 21 consecutive days. Disease progression was assessed using feed intake, body weight, stool consistency, rectal bleeding, and Disease Activity Index (DAI). Hematological parameters were also evaluated at the end of the experimental period. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test, with statistical significance set at $p < 0.05$.*

Results: *Acetic acid administration produced marked clinical deterioration in the positive-control group, characterized by reduced feed intake and body weight, diarrhea, rectal bleeding, and a substantially elevated DAI (2.72 ± 0.31). Treatment with omeprazole and apigenin individually reduced disease severity compared with the positive-control group. The combined apigenin and vitamin*

B2 treatment produced the greatest improvement, with a DAI of 0.61 ± 0.13, accompanied by improved stool consistency, reduced rectal bleeding, and near-restoration of feed intake and body weight. The combination-treated group showed an increase in body weight of 15.0 g over the experimental period, compared with a 49.3 g loss in the positive-control group. Significant differences were also observed among groups for hematological parameters, including hemoglobin, RBCs, WBCs, hematocrit, MCV, MCH, MCHC, and RDW ($p < 0.05$).

Conclusion: *Apigenin and vitamin B2 demonstrated protective effects against acetic acid-induced acute colitis in rats, with the combined treatment producing the most pronounced improvement in clinical and physiological indicators of disease severity. These findings suggest that the combination may have enhanced protective activity associated with improved disease status and hematological stability. Further studies involving larger sample sizes and mechanistic biomarkers are warranted to confirm the potential synergistic effects and underlying mechanisms of this combination.*

1. INTRODUCTION

Acute colitis is a rapidly developing inflammatory condition of the colon characterized by mucosal injury, epithelial disruption, abdominal pain, diarrhea, rectal bleeding, and infiltration of inflammatory cells into the intestinal tissue. The condition may arise due to infections, ischemia, autoimmune reactions, chemical irritation, or exposure to inflammatory agents, leading to excessive immune activation and oxidative stress in the colonic mucosa (Ko et al., 2019). Experimental models of acute colitis, particularly acetic acid-induced colitis, are widely used to mimic human intestinal inflammation because they reproduce several pathological features such as ulceration, edema, necrosis, and infiltration of neutrophils similar to those observed in inflammatory bowel disorders (Perse and Cerar, 2012). Recent studies indicate that acute colitis is associated with increased production of pro-inflammatory cytokines, reactive oxygen species, and disruption of intestinal barrier integrity, contributing to severe tissue damage and impaired gastrointestinal function (Neurath, 2019).

In this context, the oxidative stress plays a crucial role in the progression of colonic inflammation. When the generation of reactive oxygen species surpasses the ability of the body's endogenous antioxidant defense systems, oxidative stress takes place, resulting in cellular damage and thus contributing towards the occurrence of numerous inflammatory and degenerative diseases. Although endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) play a significant role in maintaining redox homeostasis, malondialdehyde (MDA) has become a recognized redox biomarker of lipid peroxidation (Liguori et al., 2018). A decrease in the antioxidant capacity stimulates inflammatory damage and exposes the mucosa (Muro et al., 2024).

Nuclear factor-kappa B (NF-κB) signalling pathway is amongst the most important molecular pathways involved in colitis development. NF-κB signaling pathway activation plays an important role in the immunopathogenesis of inflammatory bowel disease by promoting the transcription of pro-inflammatory cytokines and perpetual mucosal inflammation (Mukherjee et al., 2024). The long-lasting activation of this system is associated with ulcer formation, damage to the epithelium, and persistent inflammation. Recent studies suggest that regulating the activation of NF-κB by oxidative stress is a potential therapeutic intervention to treat IBD (Lu et al., 2022, Li et al., 2023).

Due to the recent interest in natural bioactive substances, flavonoids have become a topic of discussion as viable therapeutic agents. More attention has been attracted towards natural bioactive substances with anti-inflammatory and antioxidant properties over the last few years.

Flavonoids are a family of polyphenolic chemicals that can be found in fruits, vegetables, and medicinal plants. Apigenin is a naturally occurring flavone (strong antioxidant, anti-inflammatory, immunomodulatory) that can be found in parsley, chamomile, celery, and citrus fruits. Mechanistic research highlights that apigenin decreases the formation of ROS, increases the activity of antioxidant enzymes, and inhibits the synthesis of pro-inflammatory cytokines regulated by NF-κB (Salehi et al., 2019). Further studies showcase that apigenin improves intestinal epithelial barrier integrity as well as reduces intestinal inflammation and decreases the levels of pro-inflammatory cytokines in animal models of colitis (Li and Weigmann, 2023, Zhou et al., 2025).

Riboflavin or vitamin B2 is a water-soluble vitamin engaged in mitochondrial energy metabolism and redox mechanisms within the cell. It facilitates oxidative phosphorylation and glutathione recycling as a precursor of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) which enhance antioxidant defense capacity (Saedisomeolia and Ashoori, 2018). New research suggests that supplementation with riboflavin can change immunological reactions and reduce oxidative stress in inflammatory states. Moreover, they also discovered that riboflavin shields against intestinal inflammation by regulating the expression of cytokines and maintaining the redox balance (von Martels et al., 2019).

The current study is important because it has the potential to provide information on the therapeutic effects of natural antioxidants in inflammatory bowel disease. It aims to showcase how apigenin and vitamin B2 can alleviate oxidative stress by reducing lipid peroxidation, restoring redox balance, and improving endogenous antioxidant enzyme activity. Moreover, their role in the NF-κB-mediated production of cytokines could be investigated to reveal novel immunomodulation mechanisms that might lead to the minimization of tissue damage and the intestinal inflammation. The histology of colonic tissue will provide evidence of mucosal repair, protection and prevention of ulcers and the comparison of apigenin and riboflavin individually and in combination will showcase whether apigenin and riboflavin have synergistic anti-inflammatory and antioxidant effects (Zhang et al., 2024).

The positive findings of this study can have translational implication whereby such substances can be used as supplements to prescription drugs in order to reduce dependency on prescription drugs and reduce side effects. Additionally, by clarifying the molecular mechanisms by which these natural antioxidants regulate NF-κB signaling, oxidative stress, and inflammatory mediators, the study will close a significant research gap and aid in the creation of innovative antioxidant-based IBD treatment approaches (Fu et al., 2022, Rabinowitz et al., 2026). Thus, the current study assesses how apigenin and vitamin B2 protect albino rats from acute colitis caused by acetic acid. The study evaluates pro-inflammatory cytokines (TNF-α and IL-6), oxidative stress markers (MDA, GSH, SOD, and CAT), inflammatory markers (MPO), and histological changes in colon tissue. This study is to offer experimental proof of the therapeutic potential of apigenin and vitamin B2 in the treatment of inflammatory bowel disease by clarifying the underlying antioxidant and anti-inflammatory mechanisms (Ansari et al., 2021).

Although individual antioxidant treatments have been investigated, there isn't much experimental data on:

- The combined or relative effectiveness of vitamin B2 and apigenin in models of acute colitis.
- These substances' mechanistic regulation of inflammatory mediators (MPO, TNF-α, IL-6) and oxidative stress indicators (MDA, GSH, SOD, CAT).
- The effect of chemically induced injury on the histopathological repair of colonic tissue.

Filling in these gaps can offer vital information on new antioxidant-based IBD treatment approaches. The antioxidant effectiveness of apigenin and vitamin B2 in acetic acid-induced acute colitis has not been thoroughly studied. Their impact on oxidative stress indices and NF-κB-mediated inflammatory signaling has not yet been thoroughly evaluated mechanistically.

Objectives

- To evaluate the effects of apigenin alone and apigenin in combination with vitamin B_2 on disease activity, behavioral responses and on body-weight changes during the course of experimentally induced acute colitis.
- To determine the effects of apigenin alone and in combination with vitamin B_2 on hematological parameters, including hemoglobin concentration, red blood cell count, white blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count.

2. Material and Method

2.1 Experimental Site

The current experimental work was done at Institute of Physiology and Pharmacology under Faculty of Veterinary Science at University of Agriculture Faisalabad, Pakistan. The institute has well equipped laboratories and animal housing facilities needed to conduct experimental biomedical research including biochemical assays, histopathological evaluation and animal experimentation.

2.2 Experimental Animals

Thirty (30) healthy adult male albino Wistar rats (Rattus norvegicus) in weight range of 200-300g were utilized in the given study. Laboratory animals were sourced in the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, which has a certified breeding colony of laboratory animals.

The animal house of the Institute of Physiology and Pharmacology University of Agriculture Faisalabad, kept all animals in polypropylene cages with controlled environmental conditions. The environmental conditions were kept as follows:

- Temperature: 27 ± 2 °C
- Relative humidity: 50–60 %
- Light cycle: 12-hour light / 12-hour dark

The rats were allowed to eat the standard rat laboratory pellet diet and have fresh drinking water at ad libitum during the experiment. There was proper ventilation and hygienic conditions to make the environment conducive to the animals.

All animals were acclimatized to laboratory conditions (seven days before starting the experimental procedures) in order to familiarize them with the laboratory conditions and reduce stress. Acclimatization is an important step that stabilizes physiological parameters before the start of experimental treatments. The experimental study lasted 21 days. And all animals were randomly allocated to the experimental groups using a computer-generated random sequence into 5 groups from G_1-G_5 with 5 rats in each group. The allocation is generated automatically by a system that does not reveal future assignments.

2.3 Ethical Approval

All the animal-experimental operations were conducted in compliance with the internationally recognized guidelines of the animal laboratory use and care. The Institutional Animal Ethics Committee (IAEC) of the University of Agriculture Faisalabad (8817/ORIC) approved the experimental protocol. Maximum efforts were taken to ensure the reduction of animal suffering and stress during the experimental period. Procedures involving animals were performed under the supervision of trained personnel. The animals were humanely euthanized at the end of the experiment in order to minimize the pain and suffering.

2.4 Chemicals and Reagents

Apigenin was obtained from Swanson® Apigenin 50 mg capsules and administered orally after suspension in normal saline. Riboflavin (Vitamin B2) was obtained from NOW Foods® Vitamin B2 capsules and dissolved in distilled water before administration. Omeprazole (Risek® 20 mg) was used as the standard drug. This is particularly relevant because the study investigated omeprazole in rats with DSS-induced ulcerative colitis and used 20 mg/kg Hu et al. (2020). The authors specifically evaluated omeprazole pharmacokinetics in UC rats. Acetic acid (2%) was used for induction of experimental colitis.

2.5 Induction of Experimental Colitis

Experimental colitis was induced using the acetic acid-induced colitis model. Prior to induction, the

rats were fasted for 24 hours, while water was allowed freely. Under light anesthesia, a flexible catheter was gently inserted approximately 6–8 cm into the rectum of each rat. A 2 % solution of glacial acetic acid was slowly administered orally using a syringe. After administration, the animals were kept in a head-down posture of about 30 seconds to avoid spillage of the solution. The acetic acid produces the effects of chemical injury of the colonic mucosa such as the destruction of the epithelium, ulceration, edema and inflammatory infiltration. These pathological changes are similar to those of human ulcerative colitis.

2.6 Experimental Design

The experimental animals were randomly separated into five groups each having six rats. The use of all treatments was done in 21 consecutive days.

Table 3.1: Experimental Design to show Group Allocation, Duration and treatment regimen.

Groups	N	Days	Induction/Treatment
Group 1: NC	6	0-21	No induction, no treatment
Group 2: PC	6	0-21	2% Glacial Acetic acid given orally on alternate days
Group 3: StD	6	0-21	2% Glacial Acetic acid + Omeparazole @ (20mg/kg mixed with saline) and given as oral gavage
Group 4: Flv	6	0-21	2% Glacial Acetic acid + Apigenin @ 50mg/kg mixed with saline and given as oral gavage
Group 5: Cmplx	6	0-21	2% Glacial Acetic acid + Apigenin @ 50mg/kg + Vitamin B2 (100mg/kg) mixed in saline and given as oral gavage

Clinical Monitoring

Daily monitoring of animals during the experiment period was done to determine the progression and severity of the experimentally induced colitis. The general health condition and progression of the disease in each animal was routinely monitored using clinical observations. The parameters that were studied and recorded included the following:

Body Weight

The weight of every animal was taken at specified intervals during the study using a digital weighing balance calibrated with the known weight of an animal. Alterations in body weight were regarded as a key factor in the severity of disease since a significant reduction in weight is usually linked to inflammation in the gut, dehydration, and loss of nutrient absorption.

Feed intake

The amount of feed consumed by the animals was observed on a daily basis to assess changes in the appetite and overall physiological status of the animals. Reduction in feed intake was deemed to be a sign of systemic disease and development of intestinal inflammation.

Stool consistency

Stool consistency was examined visually and was classified as normal, soft or diarrheal. Stool consistency changes were a valuable clinical outcome in determining intestinal dysfunction and the extent of colonic inflammation.

Rectal bleeding

The rectal bleeding was measured through visual inspection and by the presence of blood in the fecal matter. The extent of bleeding was between occult and gross bleeding and it was regarded as a direct measure of mucosal damage and inflammatory destruction in the colon.

Disease Activity Index (DAI)

Based on these parameters the Disease Activity Index (DAI) a commonly accepted scoring system to measure disease severity in experimental colitis

was calculated. A numerical value was attached to each parameter, according to its severity and the total score of the DAI was determined as the mean of the separate scores. The larger DAI score showed a more severe disease as it showed more inflammation, mucosal destruction, and clinical worsening. This has been an adopted scoring system (given below) in experimental studies of ulcerative colitis to measure disease progression and treatment effectiveness (Almutary et al., 2023).

Scoring Criteria of DAI

1. **Behavioural analysis**: 0 = normal, 1 = mild lethargy, 2 = moderate lethargy, 3 = severe inactivity
2. **Stool consistency**: 0 = normal, 1 = soft, 2 = loose, 3 = diarrhoea
3. **Faecal blood**: 0 = none, 1 = occult, 2 = mild visible, 3 = gross bleeding

DAI (Per Rat Calculation)

DAI (Disease Activity Index) is typically calculated as the average of the three scores:

$$DAI = \frac{(Behavior + Stool + Blood)}{3}$$

2.8 Sample Collection

By the conclusion of the experimental time, all the animals were anesthetized with the help of the corresponding anesthetic and sacrificed in humane conditions according to the general ethical standards to reduce pain and suffering. A midline abdominal incision was made under sterile conditions and the entire colon was removed with caution beginning at the cecum and ending at the rectum. The extracted colon was put on a clean surface and rinsed with cold phosphate-buffered saline (PBS) to get fecal contents and debris off. Care was taken to avoid mechanical damage to the tissue during handling. Macroscopic parameters such as colon length, colon weight and the appearance of visible lesions that include ulceration, edema and hemorrhage were meticulously documented because they are significant measurements of the extent of colonic inflammation. Then, proper methods of serum separations and biochemical studies were applied to gather blood samples. The serum obtained was utilized in order to test the biochemical markers and pro-inflammatory cytokines. The procedure of collecting tissue and blood samples was conducted under the guidelines of the established experimental procedures that are typically performed on animal models of inflammatory bowel disease to guarantee the quality and reliability of the results (Lee et al., 2023).

2.9 Hematological Analysis (CBC)

Blood samples collected in EDTA tubes were analyzed using an automated hematology analyzer. The following hematological parameters were measured:

RBC-Related Parameters

- Hemoglobin (Hb)
- Red Blood Cell Count (RBC)
- Hematocrit (HCT)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)
- Mean Corpuscular Hemoglobin Concentration (MCHC)

WBC and Inflammatory Parameter

- White Blood Cell Count (WBC)

Platelet Parameters

- Platelet Count

Blood samples were processed according to standard laboratory procedures and analyzed using a Medonic M-Series hematology analyzer.

2.10. Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant. All statistical analyses and graphical representations were carried out using GraphPad Prism software.

Results and Discussion

3.1. Feed Intake (g/day) - Day 1 vs Day 21

Feed intake is an important physiological parameter used to assess general health status,

metabolic activity, and disease severity in experimental animals. In inflammatory conditions such as acetic acid–induced colitis, reduced feed intake is commonly observed due to abdominal discomfort, mucosal injury, systemic inflammation, and altered gut motility. Restoration of feed intake reflects recovery of gastrointestinal function and overall well-being. In the present study, feed intake was recorded on Day 1 and Day 21 across all experimental groups (Table 4.6).

The NC group showed a slight increase in feed intake from 20.2 g/day to 22.2 g/day, indicating normal growth and physiological development over the study period. In contrast, the PC group exhibited a marked reduction in feed intake (from 20.2 g/day to 12.0 g/day), reflecting severe anorexia and gastrointestinal distress caused by acetic acid–induced colitis. This reduction is consistent with inflammatory-mediated appetite suppression, where cytokines such as TNF-α and IL-1β disrupt hypothalamic appetite regulation and gastrointestinal comfort.

Table 4.2. Feed intake (g/day) in experimental groups (Day 1 vs Day 21)

Group	n	Day 1 Mean ± SD (g/day)	Day 21 Mean ± SD (g/day)	Mean Change ± SD	% Change
G1 (NC)	6	20.17 ± 0.75	22.17 ± 0.75	+2.00 ± 0.00	+9.92%
G2 (PC)	6	20.17 ± 0.75	12.00 ± 0.89	−8.17 ± 0.75	−40.50%
G3 (StD)	6	20.17 ± 0.75	17.00 ± 0.89	−3.17 ± 0.75	−15.70%
G4 (Flv)	6	20.17 ± 0.75	16.00 ± 0.89	−4.17 ± 0.75	−20.66%
G5 (Cmplx)	6	20.17 ± 0.75	19.17 ± 0.75	−1.00 ± 0.00	−4.96%

Among the treated groups, the StD group showed partial recovery with a moderate reduction in feed intake (−3.2 g/day), indicating limited protective efficacy of standard therapy. The Flv group showed a slightly higher reduction (−4.2 g/day), suggesting moderate but incomplete restoration of feeding behavior. In contrast, the Cmplx group demonstrated near-normal feed intake with only a minimal reduction (−1.0 g/day), indicating strong recovery of appetite and gastrointestinal function. The improvement observed in the combined treatment group is in agreement with previous studies reporting that flavonoids and vitamin-based antioxidants improve appetite and feeding behavior by reducing intestinal inflammation and oxidative stress. Zambrano et al. (2019) reported that inflammatory bowel conditions significantly reduce food intake due to systemic cytokine elevation, while antioxidant therapy helps restore normal feeding behavior. Similarly, Farias et al. (2020) demonstrated that flavonoid supplementation improves metabolic and gastrointestinal function, leading to increased food consumption in colitis models. Nirmaan et al. (2020) further reported that bioactive compounds reduce gut inflammation and normalize feeding patterns by improving intestinal motility and reducing discomfort. Additionally, Eshak (2018) observed that phytochemical-rich diets enhance recovery of feeding behavior through mucosal healing and reduced inflammatory burden.

3.2. Body Weight (g) — Day 1 vs Day 21

Body weight is a key indicator of overall physiological status, nutritional balance, and disease severity in experimental animals. In colitis models, body weight reduction is commonly associated with decreased feed intake, impaired nutrient absorption, intestinal inflammation, and increased metabolic demand due to systemic cytokine release. In contrast, recovery or gain in body weight reflects improved intestinal integrity and restoration of normal metabolic and absorptive functions.

In the present study, body weight was recorded on Day 1 and Day 21 across all experimental groups, showing distinct patterns of gain or loss depending on disease induction and treatment response.

Table 4.3. Body weight (g) in experimental groups (Day 1 vs Day 21)

Group	Day 1 Mean	Day 21 Mean	Change
G1 (NC)	223.3	248.2	+24.9
G2 (PC)	223.3	174.0	−49.3
G3 (StD)	223.3	206.8	−16.5
G4 (Flv)	223.3	213.3	−10.0
G5 (Cmplx)	223.3	238.3	+15.0

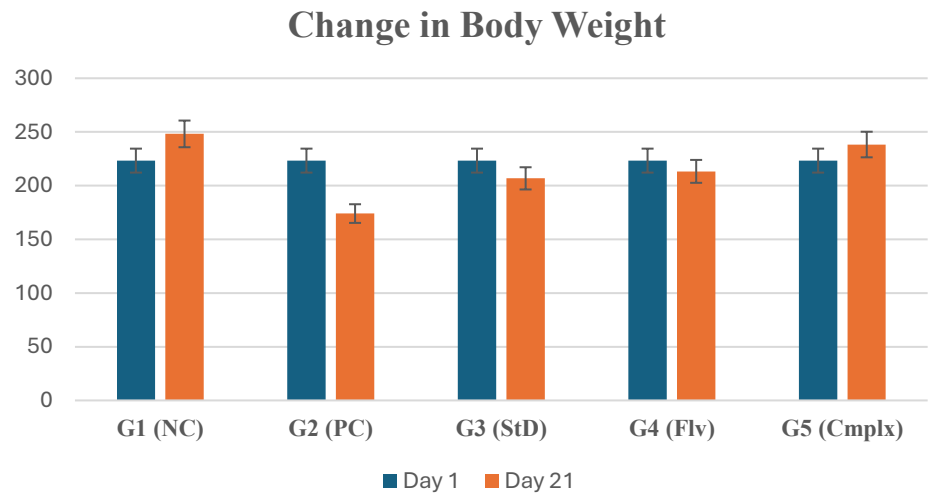


Figure 4.6. Mean body weight (g) on Day 1 and Day 21 in experimental groups

The NC group showed normal physiological growth with an increase in body weight from 223.3 g to 248.2 g, indicating healthy metabolic progression throughout the study period. In contrast, the PC group exhibited a marked reduction in body weight (223.3 g to 174.0 g), reflecting severe weight loss due to acetic acid–induced colitis. This reduction is strongly associated with inflammatory-mediated catabolism, reduced nutrient absorption, and decreased food intake.

Among treated groups, the StD group showed partial protection with moderate weight loss (−16.5 g), indicating limited recovery. The Flv group demonstrated better preservation of body weight (−10.0 g), suggesting improved protective effects of apigenin alone. In contrast, the Cmplx group showed a significant increase in body weight (+15.0 g), indicating strong recovery and restoration toward normal physiological growth. This suggests a synergistic effect of apigenin and vitamin B2 in improving nutrient utilization, reducing inflammation, and restoring metabolic balance.

Findings from previous studies also support these results. It has been reported that inflammatory bowel conditions significantly reduce body weight due to impaired nutrient absorption and systemic inflammatory stress (Zambrano et al., 2019). Flavonoid supplementation has been shown to improve weight maintenance by reducing oxidative stress and enhancing intestinal mucosal recovery (Farias et al., 2020). Antioxidant compounds also help prevent catabolic weight loss by modulating inflammatory pathways and improving gut function (Nirmaan et al., 2020), while phytochemical-based interventions enhance nutrient absorption and support growth recovery in gastrointestinal inflammatory models (Eshak, 2018).

3.3. Stool Consistency and Rectal Bleeding

Stool consistency and rectal bleeding are important clinical indicators of colonic mucosal

integrity, reflecting the severity of inflammation, epithelial damage, and impaired intestinal function in colitis. Stool consistency primarily represents the water absorption capacity and epithelial transport function of the colon, while rectal bleeding (fecal blood) indicates the extent of mucosal ulceration, vascular injury, and tissue breakdown. Disruption of epithelial tight junctions during colitis leads to increased intestinal secretion, reduced absorption, and diarrhea, whereas progressive mucosal injury results in visible blood in feces.

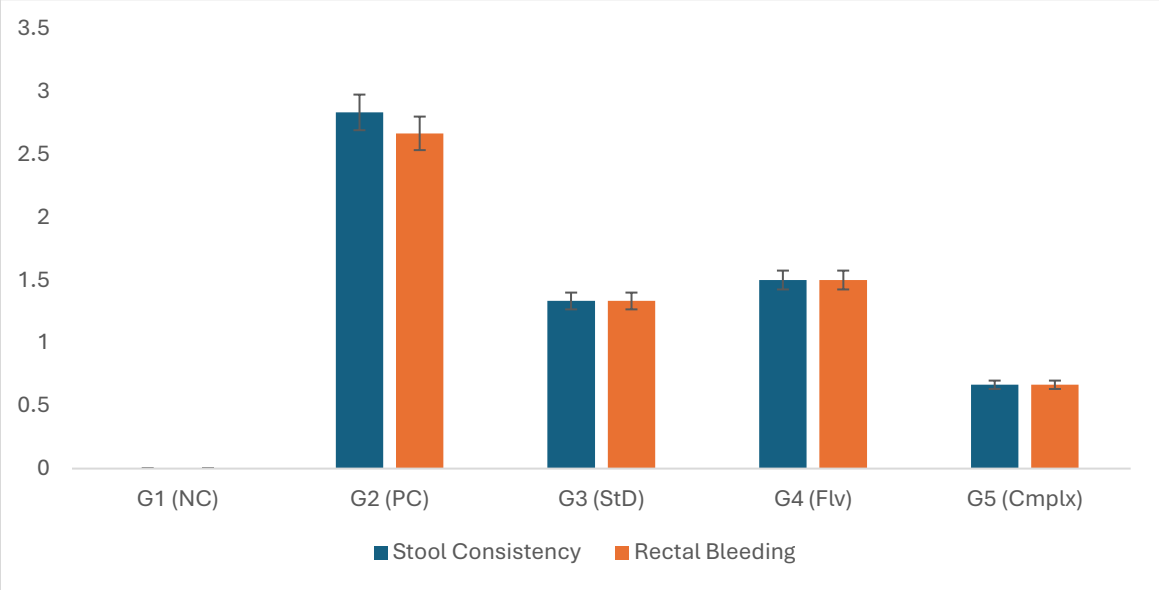


Figure 4.2. Mean stool consistency score of experimental groups

The positive control (PC) group showed severe diarrhea (2.83), indicating marked mucosal damage and loss of absorptive function, whereas the negative control (NC) group maintained normal stool consistency (0.00). All treatment groups demonstrated progressive improvement in stool form. The standard drug (StD) group reduced the score to 1.33, flavonoid-treated (Flv) group to 1.50, and the complex (Cmplx) group showed near-normal stool consistency (0.67). Overall, the combined treatment demonstrated approximately 76% improvement compared to the PC group, indicating strong restoration of intestinal absorptive and barrier function.

Similarly, rectal bleeding followed a comparable pattern of improvement across groups, reflecting the extent of mucosal healing. The PC group exhibited severe rectal bleeding (2.67), confirming extensive mucosal ulceration and vascular damage, whereas the NC group showed no bleeding. Treatment groups demonstrated a dose-dependent reduction in fecal blood scores. The StD and Flv groups showed moderate protection, while the Cmplx group demonstrated the lowest score (0.67), indicating substantial mucosal repair and reduced epithelial injury. Overall, the combined treatment reduced rectal bleeding by approximately 75% compared to the PC group.

The observed improvements in both stool consistency and rectal bleeding are consistent with previous research showing that flavonoid-rich and antioxidant compounds enhance epithelial regeneration, strengthen tight junction integrity, and reduce intestinal inflammation. Flavonoids have been reported to restore barrier function and reduce diarrhea severity through modulation of epithelial permeability (Tepe and Tepe, 2020; Farias et al., 2020). Additionally, antioxidants help regulate oxidative stress, thereby preserving vascular integrity and reducing mucosal hemorrhage (Zambrano et al., 2019). Plant-derived bioactives further support intestinal healing by stabilizing electrolyte transport and promoting mucosal repair (Nirmaan et al., 2020).

3.4. Disease Activity Index (DAI)

The Disease Activity Index (DAI) integrates behavioral changes, stool consistency, and fecal blood to provide a comprehensive measure of colitis severity and therapeutic response.

Table 4.1. Group mean DAI values

Group	Mean DAI ± SD
G1 (NC)	0.00 ± 0.00
G2 (PC)	2.72 ± 0.31
G3 (StD)	1.33 ± 0.27
G4 (Flv)	1.44 ± 0.26
G5 (Cmplx)	0.61 ± 0.13

Values are expressed as Mean ± Standard Deviation (SD). G1 (NC) = Normal Control group; G2 (PC) = Positive Control group; G3 (StD) = Standard treatment group; G4 (Flv) = Flavonoid-treated group; G5 (Cmplx) = Combined/complex treatment group. DAI = Disease Activity Index.

The PC group showed the highest DAI (2.72 ± 0.31), confirming severe colitis induction, while NC remained at baseline. Treatment groups showed progressive reduction in disease severity, with StD and Flv demonstrating moderate improvement and Cmplx showing the lowest DAI (0.61 ± 0.13), indicating near-complete recovery. The combined therapy reduced overall disease activity by approximately 78% compared to PC.

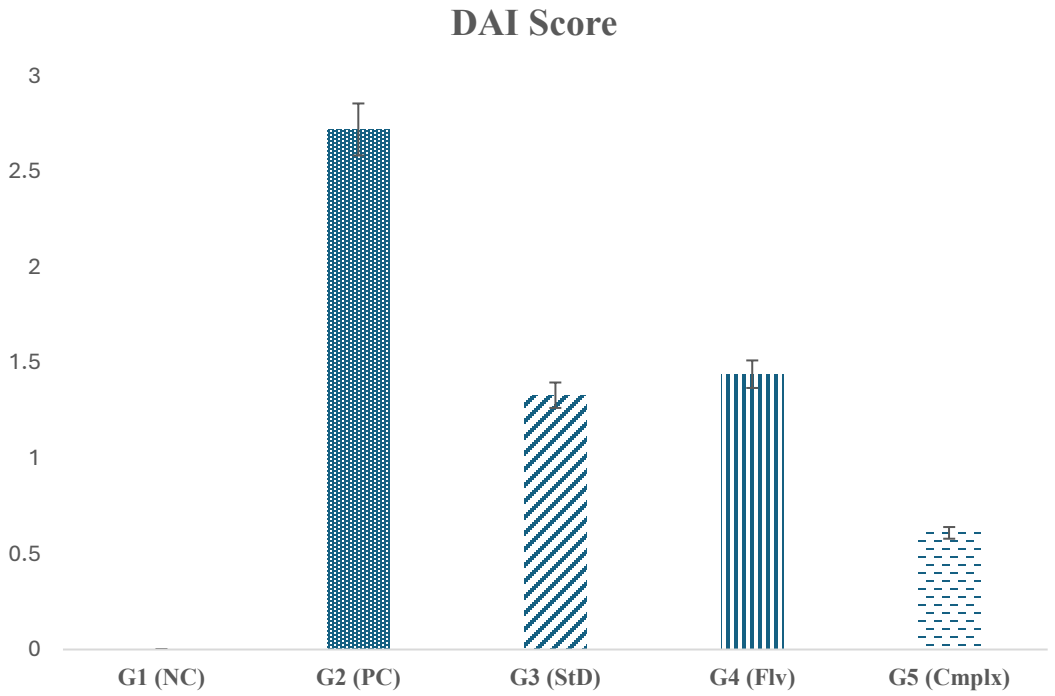


Figure 3.6. Mean Disease Activity Index (DAI) of experimental groups

These findings are consistent with reports that antioxidant therapy reduces DAI by suppressing inflammatory cytokines and oxidative stress (Zambrano et al., 2019). Flavonoids improve disease scores by enhancing epithelial regeneration and reducing inflammation (Farias et al., 2020). Similar reductions in colitis severity have been observed with phytochemical interventions

(Eshak, 2018), while dietary antioxidants consistently improve inflammatory disease indices (Tepe and Tepe, 2020).

3.6 Hematological Assessment of Rats

Hematological assessment provides crucial insight into systemic health, oxygen-carrying capacity, immune response, coagulation status, and overall physiological stability during inflammatory conditions such as colitis. The present study evaluated a complete blood profile including hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, hematocrit (HCT), and red cell indices (MCV, MCH, MCHC, RDW). These parameters collectively reflect erythropoietic activity, immune modulation, and hematological integrity under disease and treatment conditions.

Overall, the results demonstrated relatively stable hematological parameters across all experimental groups, with no major pathological deviations observed. This suggests that the induced inflammatory model and administered treatments maintained systemic hematological homeostasis.

Table 3.6. Summary of One-Way ANOVA for Hematological Parameters in Experimental Rats

Parameter	G1 (NC), Mean ± SD	G2 (PC), Mean ± SD	G3 (StD), Mean ± SD	G4 (Flv), Mean ± SD	G5 (Cmplx), Mean ± SD	F(4,25)	P-value	η^2
Hemoglobin (Hb, g/dL)	14.88 ± 0.26	10.03 ± 0.29	13.75 ± 0.19	12.75 ± 0.19	14.45 ± 0.19	428.34	<0.001	0.986
RBC ($\times 10^6$/µL)	7.65 ± 0.19	5.13 ± 0.12	6.90 ± 0.09	6.28 ± 0.15	7.38 ± 0.12	267.68	<0.001	0.977
WBC ($\times 10^3$/µL)	7.65 ± 0.19	14.92 ± 0.31	9.65 ± 0.11	11.28 ± 0.19	8.65 ± 0.11	961.93	<0.001	0.994
Platelets ($\times 10^3$/µL)	865.00 ± 11.83	612.50 ± 9.35	792.50 ± 9.87	732.50 ± 9.35	832.50 ± 7.91	606.20	<0.001	0.990
Hematocrit (HCT, %)	44.50 ± 1.05	30.50 ± 1.05	41.00 ± 0.89	38.00 ± 0.89	43.00 ± 0.63	200.05	<0.001	0.970
MCV (fL)	58.18 ± 1.28	59.43 ± 2.07	59.43 ± 0.89	60.50 ± 0.97	58.23 ± 1.08	3.23	0.029	0.341
MCH (pg)	19.47 ± 0.32	19.53 ± 0.16	19.93 ± 0.30	20.28 ± 0.37	19.57 ± 0.15	8.15	<0.001	0.566
MCHC (g/dL)	33.45 ± 0.44	32.93 ± 1.16	33.55 ± 0.27	33.57 ± 0.45	33.62 ± 0.62	0.98	0.434	0.136
RDW-CV (%)	12.65 ± 0.19	16.92 ± 0.25	13.65 ± 0.19	14.65 ± 0.19	13.17 ± 0.11	440.51	<0.001	0.986
PCT (Plateletcrit, %)	0.86 ± 0.01	0.61 ± 0.01	0.79 ± 0.01	0.73 ± 0.01	0.83 ± 0.01	639.14	<0.001	0.990
PDW (fL)	10.65 ± 0.19	14.92 ± 0.31	11.65 ± 0.11	12.65 ± 0.11	11.17 ± 0.11	440.51	<0.001	0.986

The present study demonstrated significant differences among the experimental groups for most of the evaluated hematological parameters. One-way ANOVA revealed significant between-group differences in hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), plateletcrit (PCT), and platelet

distribution width (PDW) ($p < 0.05$). In contrast, mean corpuscular hemoglobin concentration (MCHC) did not differ significantly among the experimental groups ($F(4,25) = 0.98$, $p = 0.434$). These findings indicate that experimental treatment conditions were associated with measurable differences in several hematological indices, although the magnitude and direction of these changes varied among parameters.

Hemoglobin concentration, RBC count, and hematocrit showed marked between-group differences. Hemoglobin demonstrated a highly significant overall group effect ($F(4,25) = 428.34$, $p < 0.001$, $\eta^2 = 0.986$), while RBC count ($F(4,25) = 267.68$, $p < 0.001$, $\eta^2 = 0.977$) and HCT ($F(4,25) = 200.05$, $p < 0.001$, $\eta^2 = 0.970$) also differed significantly. The disease-control group showed lower mean values for these parameters than the normal-control group, whereas the treatment groups showed comparatively greater preservation of erythrocytic parameters. These findings are compatible with hematological disturbances associated with inflammatory disease; however, the present data alone do not establish the specific mechanisms responsible for these changes.

The observed reductions in Hb, RBC count, and HCT in the disease-control group may be consistent with anemia-associated changes occurring during intestinal inflammatory conditions. In experimental colitis, such alterations may potentially arise from factors including inflammatory processes, reduced nutrient utilization, intestinal blood loss, or altered iron metabolism. However, because iron status, ferritin, transferrin saturation, reticulocyte count, and blood loss were not directly assessed in the present study, these mechanisms should be regarded as possible explanations rather than confirmed mechanisms.

The erythrocyte indices also demonstrated variable responses among the experimental groups. MCV showed a statistically significant difference among groups ($F(4,25) = 3.23$, $p = 0.029$, $\eta^2 = 0.341$), while MCH showed a significant group effect ($F(4,25) = 8.15$, $p < 0.001$, $\eta^2 = 0.566$). In contrast, MCHC did not show a statistically significant difference ($F(4,25) = 0.98$, $p = 0.434$, $\eta^2 = 0.136$). Therefore, the results do not support the previous statement that all red-cell indices were significantly altered. Instead, the findings suggest selective changes in erythrocyte size and hemoglobin content, while mean corpuscular hemoglobin concentration remained relatively comparable among groups.

RDW demonstrated a pronounced between-group difference ($F(4,25) = 440.51$, $p < 0.001$, $\eta^2 = 0.986$). The increased RDW observed in the disease-control group indicates greater variability in erythrocyte size, which may be compatible with altered erythropoietic dynamics during inflammatory conditions. Nevertheless, RDW is a nonspecific hematological marker, and the present study cannot establish whether the observed variation resulted specifically from iron deficiency, oxidative stress, inflammation, or another mechanism in the absence of additional biochemical and hematological measurements.

WBC count showed a significant difference among the experimental groups ($F(4,25) = 961.93$, $p < 0.001$, $\eta^2 = 0.994$). The disease-control group demonstrated a higher mean WBC count than the normal-control group, while comparatively lower values were observed in the treatment groups. The elevation in WBC count in the disease-control animals is consistent with an inflammatory response associated with experimental colitis. Increased circulating leukocytes may reflect systemic immune activation accompanying local intestinal inflammation. However, differential leukocyte counts and inflammatory cytokines were not evaluated in the present analysis; therefore, specific immune-cell contributions cannot be determined.

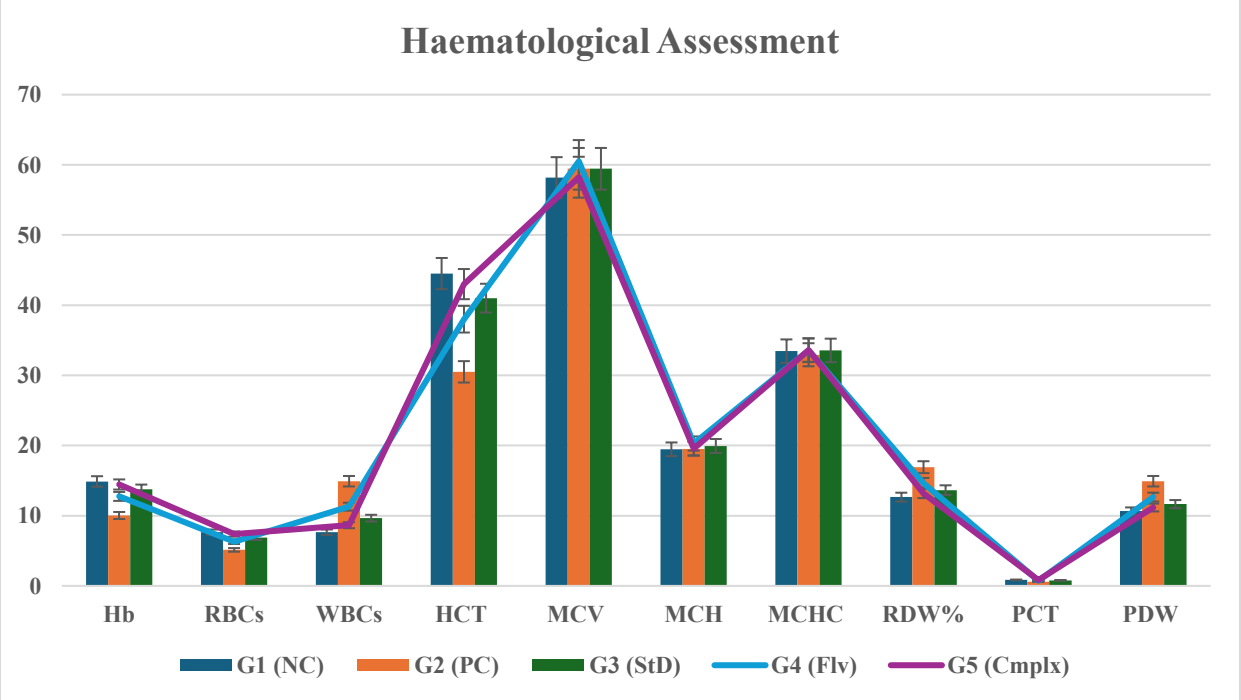
Platelet-related parameters also differed significantly among the experimental groups. Platelet count showed a significant group effect ($F(4,25) = 606.20$, $p < 0.001$, $\eta^2 = 0.990$). Similarly, plateletcrit (PCT) demonstrated a significant difference ($F(4,25) = 639.14$, $p < 0.001$, $\eta^2 = 0.990$), while platelet distribution width (PDW) also differed significantly among groups ($F(4,25) = 440.51$, $p < 0.001$, $\eta^2 = 0.986$). These findings indicate that the experimental conditions were associated with changes in platelet quantity and platelet-related indices. Because platelets participate in both hemostasis and inflammatory

processes, alterations in platelet parameters may accompany inflammatory tissue injury. Nevertheless, the present study does not allow a direct determination of whether these changes were caused by inflammatory activation, altered hematopoiesis, tissue injury, or other factors.

Taken together, the hematological findings demonstrate that experimental colitis was associated with substantial differences in several systemic hematological parameters. The most pronounced effects were observed for Hb, RBC count, WBC count, platelet count, HCT, RDW, PCT, and PDW, as reflected by their large eta-squared effect sizes. The treatment groups generally demonstrated values that were intermediate between the disease-control and normal-control groups for several parameters, suggesting a potential improvement or partial normalization following treatment. However, these observations should be interpreted descriptively until the appropriate post-hoc comparisons are considered.

The combination of altered erythrocytic, leukocytic, and platelet parameters indicates that the experimental condition was associated with changes extending beyond the local intestinal environment. Such hematological alterations are broadly compatible with the systemic consequences of inflammatory conditions. Previous literature has similarly described disturbances in erythrocyte parameters, leukocyte profiles, and platelet responses in inflammatory and intestinal disease. However, comparisons with previous studies should be interpreted cautiously because differences in animal species, disease-induction protocols, treatment duration, dose, and experimental conditions may influence hematological responses.

The present findings therefore support the utility of hematological profiling as an accompanying indicator of physiological changes in experimental colitis. Nevertheless, hematological parameters should not be considered a standalone measure of disease severity or therapeutic efficacy. Their interpretation would be strengthened by integration with histopathological findings, disease-activity scores, inflammatory biomarkers, oxidative-stress markers, and other prespecified outcomes.



Conclusion

The present study demonstrated that acetic acid-induced acute colitis produced marked clinical and physiological disturbances in rats, including reduced feed intake and body weight, diarrhea, rectal bleeding, and increased Disease Activity Index (DAI). Administration of apigenin and vitamin B2 individually provided protective effects against these disease-associated changes. However, the combined administration of apigenin (50 mg/kg) and vitamin B2 (100 mg/kg) produced the most pronounced improvement, as reflected by the lowest DAI score, improved stool consistency and reduced rectal bleeding, together with better preservation of feed intake and body weight. The combination-treated group also demonstrated significant between-group differences in most evaluated hematological parameters, including Hb, RBC count, WBC count, platelet count, HCT, MCV, MCH, RDW, PCT, and PDW. MCHC did not show a statistically significant difference among groups. The observed hematological changes indicate that the experimental condition was associated with systemic physiological alterations involving erythrocytic, leukocytic, and platelet-related parameters. The comparatively improved hematological profiles observed in the treatment groups may indicate a potential beneficial effect of the interventions. Overall, the findings indicate that apigenin combined with vitamin B2 has promising protective potential against chemically induced acute colitis in rats. Nevertheless, the apparent enhanced effect of the combination should be interpreted cautiously as evidence of improved efficacy rather than confirmed synergism, since formal interaction or synergy analyses were not performed. Further studies with larger sample sizes, comprehensive biochemical, inflammatory, oxidative-stress, and histopathological assessments are required to establish the underlying mechanisms and confirm the therapeutic potential of this combination.

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