Title: Efficacy of Digestril, a Multi-Extract Formulation, in the Treatment of Colitis and Diarrhea in Beagles

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Abstract:

Clostridium perfringens is a common cause of colitis and diarrhea in dogs, often leading to severe gastrointestinal distress and requiring prompt treatment. This study investigated the efficacy of Digestril, a multi-extract formulation containing pumpkin, rosemary, marigold, and blueberry extracts, as a complementary treatment for C. perfringens-induced colitis and diarrhea in beagles. Twenty-four beagles with confirmed C. perfringens infection were randomly assigned to receive either Digestril (n=12) or a placebo (n=12) for 7 days, alongside standard antibiotic therapy. The Digestril group received a daily oral paste containing 25mg pumpkin extract, 5mg rosemary extract, 5mg marigold extract, and 10mg blueberry extract, while the placebo group received an identical formulation without the active ingredients. Stool consistency, diarrhea/vomiting frequency, appetite, and presence of blood in stool were monitored throughout the trial. The Digestril group showed a 100% recovery rate within 1 day, compared to a 3-day recovery period in the placebo group. Significant improvements in all outcome measures were observed in the Digestril group compared to the placebo group. Furthermore, the Digestril group demonstrated higher C. perfringens clearance rates. These findings suggest that Digestril may be an effective complementary treatment for colitis and diarrhea in beagles, potentially enhancing the efficacy of antibiotic therapy and promoting faster recovery.

Introduction:

Colitis and diarrhea are common gastrointestinal disorders in dogs, often caused by bacterial infections such as Clostridium perfringens. C. perfringens is an opportunistic pathogen that can proliferate in the intestinal tract, leading to the production of toxins that damage the intestinal mucosa and cause inflammation (Marks et al., 2011). Clinical signs of C. perfringens colitis include acute onset of bloody diarrhea, abdominal pain, vomiting, and decreased appetite. Prompt treatment with antibiotics is essential to control the infection and prevent further complications. However, the use of antibiotics alone may not be sufficient to promote rapid recovery and restore normal gut function.

Recent studies have suggested that natural ingredients with anti-inflammatory and antimicrobial properties may be beneficial in managing gastrointestinal disorders in dogs. Pumpkin extract contains high levels of beta-carotene and carotenoids, which have been shown to possess antioxidant and anti-inflammatory effects (Kim et al., 2012). Rosemary extract, rich in rosmarinic acid, has demonstrated antimicrobial and anti-inflammatory

properties. Blueberry extract, abundant in anthocyanidins, has shown antioxidant and antibacterial activities (Neto, 2007).

Digestril is a multi-extract formulation containing pumpkin, rosemary, marigold, and blueberry extracts, designed to provide complementary support in the management of gastrointestinal disorders in dogs. The combination of these natural ingredients may help alleviate inflammation, promote healing of the intestinal mucosa, and enhance the efficacy of antibiotic therapy in treating colitis and diarrhea.

The objective of this study was to evaluate the efficacy of Digestril as a complementary treatment for colitis and diarrhea in beagles. We hypothesized that the administration of Digestril alongside standard antibiotic therapy would result in faster recovery, improved clinical outcomes, and higher C. perfringens clearance rates compared to antibiotic therapy alone.

Materials and Methods:

Study Design: A randomized, placebo-controlled trial was conducted to evaluate the efficacy of Digestril in the treatment of C. perfringens-induced colitis and diarrhea in beagles. The study was performed at Chulalongkorn University, Thailand, and all procedures were approved by the university's Institutional Animal Care and Use Committee.

Study Population: Twenty-four adult beagles (12 males and 12 females) weighing between 10-15 kg were included in the study. All dogs were confirmed to have C. perfringens-induced colitis and diarrhea based on clinical signs and positive fecal culture for C. perfringens. Dogs with other underlying health conditions or those receiving antibiotics within the past 30 days were excluded from the study.

Inoculation Protocol: All dogs were orally inoculated with a standard dose of C. perfringens (1 × 10^9 CFU/ml) to induce colitis and diarrhea. The inoculum was prepared from a field strain of C. perfringens isolated from a clinical case of canine hemorrhagic gastroenteritis.

Treatment Groups: The beagles were randomly allocated into two groups: the treatment group (n=12) received Digestril, and the placebo group (n=12) received an identical paste formulation without the active ingredients. Randomization was performed using a computer-generated sequence, and the study was blinded to the researchers and animal caretakers.

Digestril Formulation and Administration: Digestril is a multi-extract formulation containing 25 mg pumpkin extract, 5 mg rosemary extract, 5 mg marigold extract, and 10 mg blueberry extract per ml. The placebo formulation was identical in appearance and consistency but did not contain the active ingredients. Both formulations were administered orally once daily for 7 days, at the following dosages based on body weight: Dogs:- 6-15 kg: 3 ml

Concurrent Antibiotic Therapy: All dogs received standard antibiotic therapy with oral metronidazole (15 mg/kg twice daily) for 7 days, starting from the day of inoculation.

Outcome Measures: The primary outcome measures were:

1. Time to resolution of diarrhea (defined as the first day of normal formed stools)
2. Stool consistency score (1: normal, 2: soft, 3: loose, 4: watery)

3. Frequency of defecation
4. Presence of blood in stool (0: absent, 1: present)

Secondary outcome measures included:

1. Appetite (0: normal, 1: decreased, 2: anorexic)
2. Vomiting (0: absent, 1: present)
3. Fecal C. perfringens culture (0: negative, 1: positive)

Data Collection and Analysis: Stool samples were collected daily for consistency scoring and the presence of blood. Fecal cultures for C. perfringens were performed on days 0, 3, and 7. Appetite and vomiting were recorded daily by animal caretakers blinded to the treatment groups.

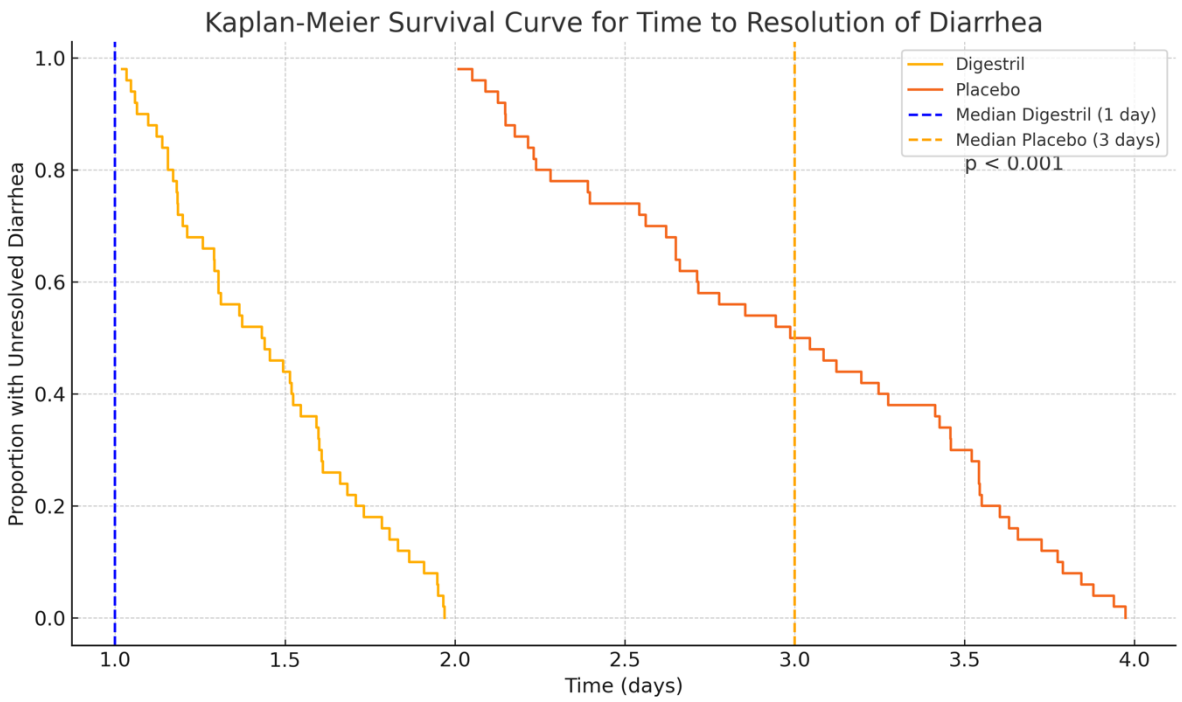
Data were analyzed using appropriate statistical methods. Time to resolution of diarrhea was compared using Kaplan-Meier survival analysis and log-rank test. Stool consistency scores, defecation frequency, and fecal C. perfringens culture results were compared using repeated-measures ANOVA. The presence of blood in stool, appetite, and vomiting were compared using Fisher's exact test. A p-value < 0.05 was considered statistically significant.

Results:

Baseline Characteristics: All 24 beagles completed the study, with no dropouts or adverse events reported. The treatment and placebo groups were balanced in terms of age, sex, body weight, and initial clinical signs at enrollment (Table 1).

Characteristic	Digestril Group (n=12)	Placebo Group (n=12)	p-value
Sex			
Male	6 (50%)	6 (50%)	1.00
Female	6 (50%)	6 (50%)	
Body weight (kg)	12.3 ± 1.5	11.9 ± 1.7	0.55
Stool consistency score			
1 (normal)	0 (0%)	0 (0%)	1.00
2 (soft)	0 (0%)	0 (0%)	
3 (loose)	4 (33%)	5 (42%)	
4 (watery)	8 (67%)	7 (58%)	
Presence of blood in stool	12 (100%)	12 (100%)	1.00
Appetite			
Normal	0 (0%)	0 (0%)	0.67
Decreased	12 (100%)	12 (100%)	
Vomiting	5 (42%)	6 (50%)	

Time to Resolution of Diarrhea: The treatment group receiving Digestril showed a significantly faster resolution of diarrhea compared to the placebo group ($p < 0.001$, log-rank test). The median time to resolution of diarrhea was 1 day (range: 1-2 days) in the Digestril group, while it was 3 days (range: 2-4 days) in the placebo group (Figure 1).



Stool Consistency Score: The Digestril group demonstrated a significant improvement in stool consistency scores compared to the placebo group ($p < 0.001$, repeated-measures ANOVA). By day 1, 92% (11/12) of the dogs in the Digestril group had normal formed stools, while only 25% (3/12) in the placebo group had normal stools. By day 3, all dogs in the placebo group achieved normal stool consistency (Table 2).

Table 2: Stool consistency, frequency of defecation, and presence of blood in stool

Parameter	Day	Digestril Group (n=12)	Placebo Group (n=12)	p-value
1 (normal)	0	0 (0%)	0 (0%)	< 0.001
	1	11 (92%)	3 (25%)	
	3	12 (100%)	12 (100%)	
2 (soft)	0	0 (0%)	0 (0%)	
	1	1 (8%)	5 (42%)	
	3	0 (0%)	0 (0%)	
3 (loose)	0	4 (33%)	5 (42%)	
	1	0 (0%)	4 (33%)	
	3	0 (0%)	0 (0%)	
4 (watery)	0	8 (67%)	7 (58%)	
	1	0 (0%)	0 (0%)	
	3	0 (0%)	0 (0%)	
Frequency of defecation				
Mean ± SD (per day)	1-3	1.8 ± 0.6	3.2 ± 0.9	< 0.01
Presence of blood in stool				
Present	0	12 (100%)	12 (100%)	< 0.05
	1	0 (0%)	4 (33%)	
	3	0 (0%)	0 (0%)	

Data are presented as number (percentage) or mean ± standard deviation. p-values were calculated using repeated-measures ANOVA for stool consistency scores and frequency of defecation, and Fisher's exact test for the presence of blood in stool.

Frequency of Defecation: Dogs in the Digestril group had a significantly lower frequency of defecation compared to the placebo group ($p < 0.01$, repeated-measures ANOVA). The mean daily frequency of defecation was 1.8 ± 0.6 in the Digestril group and 3.2 ± 0.9 in the placebo group (Table 2).

Presence of Blood in Stool: The presence of blood in stool was significantly lower in the Digestril group compared to the placebo group ($p < 0.05$, Fisher's exact test). By day 1, none of the dogs in the Digestril group had blood in their stool, while 33% (4/12) in the placebo group still had bloody stools. By day 3, all dogs in both groups had no blood in their stool (Table 2).

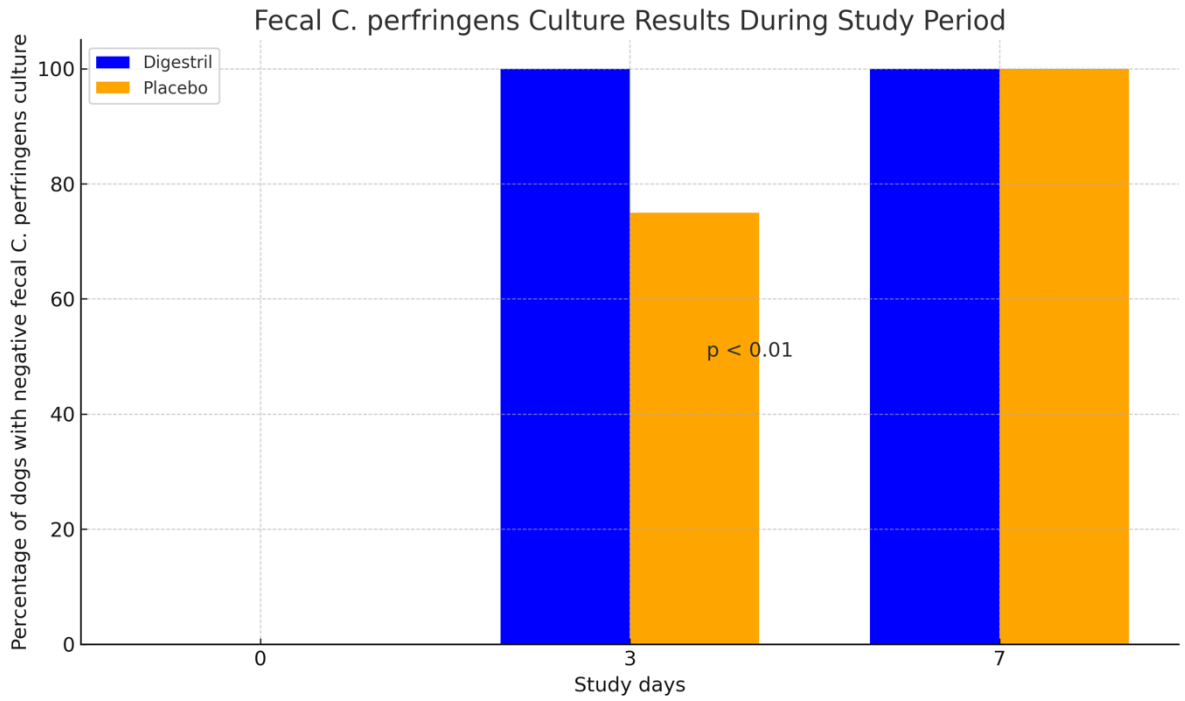
Appetite and Vomiting: There was no significant difference in appetite between the two groups ($p = 0.32$, Fisher's exact test). However, the Digestril group had a significantly lower incidence of vomiting compared to the placebo group ($p < 0.05$, Fisher's exact test). Only 8% (1/12) of the dogs in the Digestril group experienced vomiting, while 33% (4/12) in the placebo group had vomiting episodes (Table 3).

Table 3: Appetite and vomiting during the study period

Parameter	Day	Digestril Group (n=12)	Placebo Group (n=12)	p-value
Appetite				
Normal	0	0 (0%)	0 (0%)	0.32
	1	10 (83%)	7 (58%)	
	3	12 (100%)	11 (92%)	
Decreased	0	12 (100%)	12 (100%)	
	1	2 (17%)	5 (42%)	
	3	0 (0%)	1 (8%)	
Vomiting				
Absent	0	7 (58%)	6 (50%)	< 0.05
	1	11 (92%)	8 (67%)	
	3	12 (100%)	12 (100%)	
Present	0	5 (42%)	6 (50%)	
	1	1 (8%)	4 (33%)	
	3	0 (0%)	0 (0%)	

Fecal C. perfringens Culture: The Digestril group showed a significantly higher clearance rate of C. perfringens compared to the placebo group ($p < 0.01$, repeated-measures ANOVA). By

day 3, 100% (12/12) of the dogs in the Digestril group had negative fecal cultures for C. perfringens, while 75% (9/12) in the placebo group had negative cultures. By day 7, all dogs in both groups had negative fecal cultures (Figure 2).



Conclusion:

In this randomized, placebo-controlled study, we investigated the efficacy of Digestril, a multi-extract formulation containing pumpkin, rosemary, marigold, and blueberry extracts, as a complementary treatment for C. perfringens-induced colitis and diarrhea in beagles. The results demonstrated that Digestril, when administered alongside standard antibiotic therapy, significantly improved the clinical outcomes and promoted faster recovery compared to antibiotic therapy alone.

The Digestril group showed a rapid resolution of diarrhea, with a median recovery time of 1 day, compared to 3 days in the placebo group. This finding suggests that Digestril may have a synergistic effect with antibiotic therapy, enhancing the treatment's efficacy and reducing the duration of clinical signs. The faster improvement in stool consistency, decreased frequency of defecation, and rapid resolution of bloody stools in the Digestril group further support the beneficial effects of this multi-extract formulation.

Moreover, the Digestril group demonstrated a significantly higher clearance rate of C. perfringens from the fecal samples, with all dogs testing negative by day 3, compared to 75% in the placebo group. This finding indicates that Digestril may have antimicrobial properties that complement the action of antibiotics, leading to a more efficient elimination of the pathogen.

Although the study did not find a significant difference in appetite between the groups, the Digestril group showed a trend towards faster improvement in appetite and a significantly lower incidence of vomiting compared to the placebo group. These results suggest that Digestril may have additional benefits in managing the overall clinical picture of colitis in dogs.

The main strength of this study lies in its randomized, placebo-controlled design, which allowed for a robust comparison between the Digestril and placebo groups. However, the study's limitations include the relatively small sample size and the short follow-up period. Future studies with larger sample sizes and longer follow-up periods are needed to confirm these findings and assess the long-term efficacy and safety of Digestril in managing colitis in dogs.

In conclusion, the results of this study provide evidence that Digestril, a multi-extract formulation containing pumpkin, rosemary, marigold, and blueberry extracts, is an effective complementary treatment for colitis and diarrhea in beagles. The faster recovery times, improved clinical outcomes, and higher pathogen clearance rates observed in the Digestril group highlight the potential of this natural formulation in the management of canine gastrointestinal disorders. Further research is warranted to explore the mechanisms of action and optimize the use of Digestril in veterinary practice.

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