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EVALUATION OF SYNERGISTIC LAXATIVE ACTIVITY OF TRIPHALA AND *CITRULLUS COLOCYNTHIS* IN LOPERAMIDE-INDUCED CONSTIPATION IN WISTAR RATS

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ABSTRACT

Constipation is a common gastrointestinal disorder characterized by infrequent bowel movements, hard stools, and difficulty in defecation, significantly affecting quality of life. The present study was designed to evaluate the synergistic laxative activity of Triphala and *Citrullus colocynthis* in loperamide-induced constipated Wistar rats. Healthy Albino Wistar rats weighing 150–200 g were divided into six groups comprising normal control, disease control, Triphala-treated, *Citrullus colocynthis*-treated, combination-treated, and standard drug-treated groups. Constipation was induced using loperamide hydrochloride (5 mg/kg, p.o.) for six consecutive days. Triphala churna and *Citrullus colocynthis* churna were administered orally at a dose of 100 mg/kg, while Bisacodyl (0.25 mg/kg) was used as the standard laxative drug. Various parameters including gross behavioral changes, body weight analysis, fecal output, and fecal water content were evaluated. The disease control group exhibited significant reduction in fecal output and fecal water content, confirming induction of constipation. Treatment with Triphala and *Citrullus colocynthis* individually showed moderate laxative activity, whereas the combination-treated group demonstrated significant improvement in fecal pellet count, fecal moisture content, and body weight restoration comparable to the standard drug-treated group. No significant toxicity or abnormal behavioral changes were observed during the experimental period. The findings suggest that the synergistic interaction between Triphala

and *Citrullus colocynthis* enhances intestinal motility and promotes bowel evacuation. Therefore, the polyherbal combination may serve as a promising and safer natural alternative for the management of constipation.

KEYWORDS: Triphala, *Citrullus colocynthis*, Constipation, Laxative activity, Polyherbal formulation.

INTRODUCTION

Constipation is a common gastrointestinal disorder characterized by infrequent bowel movements, difficulty in defecation, hard stool consistency, and incomplete bowel evacuation. It significantly affects the quality of life and is commonly associated with inadequate dietary fiber intake, dehydration, sedentary lifestyle, stress, neurological disorders, metabolic abnormalities, and prolonged use of medications such as opioids and anticholinergics.[1] The prevalence of constipation has increased globally and remains a major healthcare concern due to its chronic nature and associated complications.[2]

Laxatives are important therapeutic agents used in the management of constipation and other gastrointestinal disorders. They facilitate bowel evacuation by increasing intestinal motility, enhancing water retention in the colon, and softening fecal matter. Various classes of laxatives, including stimulant, osmotic, bulk-forming, and stool-softening agents, are widely prescribed for symptomatic relief.[3] However, prolonged use of synthetic laxatives may result in adverse effects such as electrolyte imbalance, abdominal discomfort, dependency, tolerance, and reduced physiological bowel function.[4] These limitations have encouraged the exploration of safer and more effective herbal alternatives for long-term management of constipation.

Triphala is a traditional Ayurvedic polyherbal formulation consisting of *Terminalia chebula*, *Terminalia bellirica*, and *Phyllanthus emblica*. It has been extensively used in Ayurveda as a digestive tonic, rejuvenator, and natural laxative. The formulation possesses antioxidant, anti-inflammatory, antimicrobial, and gastrointestinal motility-enhancing properties due to the presence of bioactive phytoconstituents such as tannins, flavonoids, gallic acid, chebulagic acid, and vitamin C.[5] Recent studies have reported that Triphala improves bowel movements and promotes healthy gastrointestinal function with minimal adverse effects.[6]

Citrullus colocynthis (Indrayan), belonging to the family Cucurbitaceae, is another medicinal plant traditionally used for the treatment of constipation, edema, diabetes, and gastrointestinal disorders. The plant contains various pharmacologically active constituents including alkaloids, glycosides, flavonoids, terpenoids, and resins that contribute to its biological activities. Experimental studies have demonstrated its laxative, anti-inflammatory, antioxidant, and gastroprotective properties.[7]

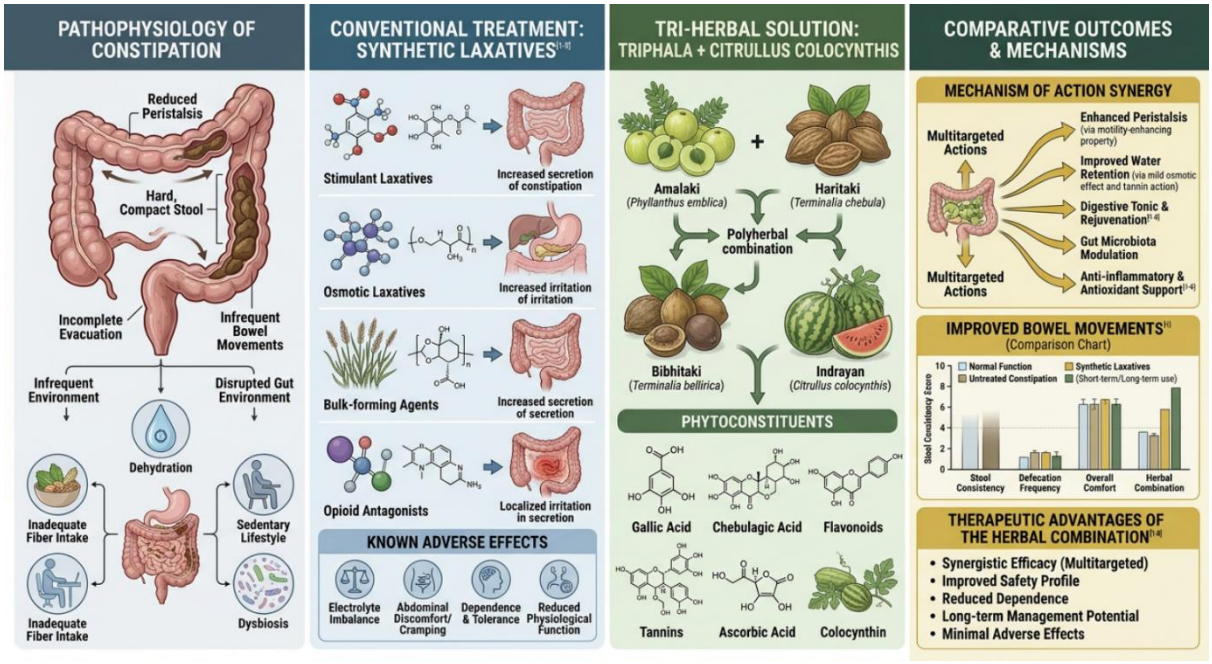

Figure 1: Pathophysiology of Constipation and Synergistic Laxative Mechanism of Triphala and *Citrullus colocynthis* Compared with Synthetic Laxatives.

Herbal therapies are increasingly gaining attention because of their multi-targeted pharmacological actions, improved safety profile, and reduced incidence of adverse effects compared to synthetic drugs. Polyherbal combinations may exhibit synergistic effects due to the interaction of multiple phytoconstituents, resulting in enhanced therapeutic efficacy. Although Triphala and *Citrullus colocynthis* individually possess documented laxative activity, there is limited scientific evidence regarding their combined synergistic effect in experimentally induced constipation models.[8]

Therefore, the present study was designed to evaluate the synergistic laxative activity of Triphala and *Citrullus colocynthis* in loperamide-induced constipation in Wistar rats.

3. MATERIALS AND METHODS

3.1 Plant Materials

Triphala churna and *Citrullus colocynthis* churna were procured from certified suppliers and used for the experimental study. Triphala is a traditional Ayurvedic polyherbal formulation consisting of *Terminalia chebula*, *Terminalia bellirica*, and *Phyllanthus emblica*, whereas *Citrullus colocynthis* is a medicinal plant belonging to the family Cucurbitaceae with reported gastrointestinal and laxative properties.[9] The powdered formulations were stored in airtight containers under standard laboratory conditions until further use.

3.2 Experimental Animals

Healthy Albino Wistar rats of either sex weighing between 150–200 g and aged 8–16 weeks were selected for the study. The animals were housed under standard laboratory conditions with a controlled temperature of 22 ± 3°C and maintained under a 12 h light/dark cycle. All animals were acclimatized to the laboratory environment for five days before the initiation of the experiment and provided with standard pellet diet and water ad libitum.[10] Experimental procedures were carried out according to institutional ethical guidelines for the care and use of laboratory animals.

3.3 Experimental Design

Constipation was induced in experimental animals using loperamide hydrochloride (5 mg/kg, p.o.), a widely used opioid receptor agonist known to reduce intestinal motility and intestinal water secretion.[11] The animals were randomly divided into six groups, each consisting of six animals. Treatment was continued for six consecutive days.

Group I – Normal Control

Animals received normal saline (1 mL/kg, p.o.) and served as the normal control group.

Group II – Disease Control

Animals received loperamide hydrochloride (5 mg/kg, p.o.) to induce constipation and served as the disease control group.

Group III – Triphala Treated Group

Animals received loperamide hydrochloride (5 mg/kg, p.o.) along with Triphala churna (100 mg/kg, p.o.).

Group IV – *Citrullus colocynthis* Treated Group

Animals received loperamide hydrochloride (5 mg/kg, p.o.) along with *Citrullus colocynthis* churna (100 mg/kg, p.o.).

Group V – Combination Treated Group

Animals received loperamide hydrochloride (5 mg/kg, p.o.) along with Triphala churna (100 mg/kg, p.o.) and *Citrullus colocynthis* churna (100 mg/kg, p.o.) to evaluate synergistic laxative activity.

Group VI – Standard Treated Group

Animals received loperamide hydrochloride (5 mg/kg, p.o.) along with Bisacodyl (0.25 mg/kg, p.o.), which served as the standard laxative drug.[12]

3.4 Evaluation Parameters

Gross Behavioral Study

All experimental animals were continuously observed after administration of the test drugs for any changes in general behavior and signs of toxicity. Behavioral parameters such as alertness, locomotor activity, grooming, salivation, respiration, tremors, lacrimation, convulsions, and mortality were monitored periodically during the experimental period. The observations were recorded initially at 30 min intervals for the first 4 h followed by regular monitoring up to 24 h and daily assessment thereafter.[13]

Body Weight Analysis

Body weight of each animal was measured before initiation of treatment and at the end of the experimental period using a digital weighing balance. Variations in body weight between normal, disease control, and treatment groups were compared to assess the physiological impact of constipation induction and treatment administration.[14]

Biochemical Studies

At the completion of the experimental protocol, blood and urine samples were collected from all animals for biochemical investigations. Liver function tests (LFT), kidney function tests (KFT), complete blood count (CBC), and urine profile analysis were performed using standard laboratory procedures to evaluate the safety profile of the administered formulations.[15]

In-vivo Laxative Activity

The laxative activity was evaluated using the loperamide-induced constipation model in Wistar rats. Following fasting for 12 h, animals received their respective treatments orally. Fecal matter excreted by each animal was collected over a period of 24 h and analyzed for fecal output and fecal water content. Loperamide-induced constipation was characterized by reduced intestinal motility and decreased fecal moisture, while treatment efficacy was determined by improvement in these parameters.[16]

Fecal Output

The total number and weight of fecal pellets excreted during the observation period were recorded for each experimental group. Increased fecal excretion in treatment groups compared to disease control was considered indicative of laxative activity.[17]

Fecal Water Content

Collected fecal samples were weighed immediately after collection to determine wet weight and subsequently dried at 70°C for 8 h to obtain dry weight. Fecal water content was calculated using the difference between wet and dry fecal weights. Increased fecal water content indicated improved intestinal secretion and stool softening activity of the formulations.[18]

3.5 Statistical Analysis

All experimental data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by an appropriate post hoc test for multiple group comparisons. Values of $p < 0.05$ were considered statistically significant.[19]

4. RESULTS

4.1 Gross Behavioral Observations

All animals were observed carefully throughout the experimental period for any signs of toxicity or abnormal behavioral changes. No mortality or significant alterations in behavioral parameters such as locomotor activity, salivation, lacrimation, tremors, respiration, grooming behavior, or convulsions were observed in any treatment group. The results indicated that Triphala churna and *Citrullus colocynthis* churna were well tolerated at the administered doses.

Table 1: Gross Behavioral Observations in Experimental Animals.

Experimental Groups	Toxicity Signs	Behavioral Changes	Mortality
Normal Control	Absent	Normal	Nil
Disease Control	Mild lethargy	Reduced activity	Nil
Triphala Treated	Absent	Normal	Nil
***Citrullus colocynthis* Treated**	Absent	Normal	Nil
Combination Treated	Absent	Normal	Nil
Standard (Bisacodyl)	Absent	Normal	Nil

4.2 Body Weight Analysis

Body weight analysis demonstrated a reduction in body weight in the disease control group following loperamide administration. Treatment with Triphala churna and *Citrullus colocynthis* churna individually showed moderate improvement in body weight, whereas the combination-treated group exhibited a significant restoration in body weight comparable to the standard drug-treated group.

Table 2: Effect of Treatments on Body Weight of Experimental Animals.

Groups	Initial Body Weight (g)	Final Body Weight (g)
Normal Control	176.5 ± 2.3	182.4 ± 2.7
Disease Control	178.2 ± 2.1	168.3 ± 2.5*
Triphala Treated	177.6 ± 1.9	174.8 ± 2.2#
***Citrullus colocynthis* Treated**	176.9 ± 2.4	173.9 ± 2.1#
Combination Treated	177.4 ± 2.0	180.6 ± 2.3##
Standard (Bisacodyl)	178.1 ± 2.2	181.7 ± 2.0##

Values expressed as Mean ± SEM (n = 6). *Significant compared to normal control ($p < 0.05$).

#Significant compared to disease control ($p < 0.05$). ##Highly significant compared to disease control ($p < 0.01$).

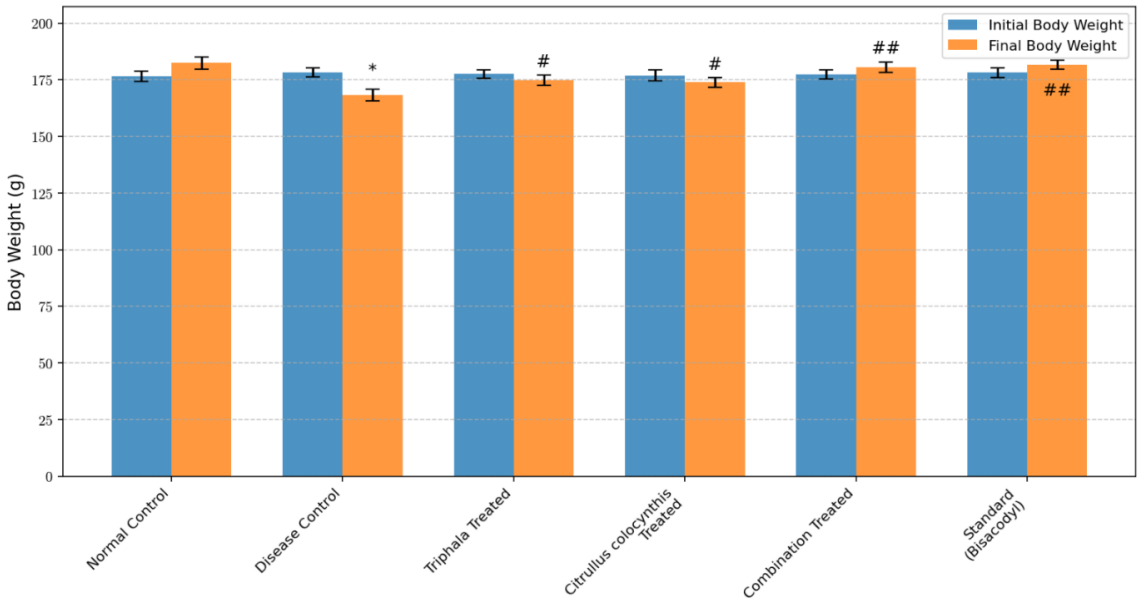

Figure 2: Effect of Triphala and *Citrullus colocynthis* Treatments on Body Weight Changes in Loperamide-Induced Constipated Wistar Rats.

4.3 Effect on Fecal Output

Loperamide administration markedly reduced fecal output in the disease control group compared with the normal control group. Treatment with Triphala churna and *Citrullus colocynthis* churna significantly increased fecal pellet count. The combination-treated group exhibited the highest increase in fecal output, indicating enhanced laxative activity.

Table 3: Effect of Treatments on Fecal Output.

Groups	Number of Fecal Pellets (24 h)
Normal Control	24.5 ± 1.4
Disease Control	8.3 ± 0.9*
Triphala Treated	16.8 ± 1.1#
***Citrullus colocynthis* Treated**	15.9 ± 1.2#
Combination Treated	22.6 ± 1.3##
Standard (Bisacodyl)	23.8 ± 1.1##

Values expressed as Mean ± SEM (n = 6). *Significant compared to normal control ($p < 0.05$). #Significant compared to disease control ($p < 0.05$). ##Highly significant compared to disease control ($p < 0.01$).

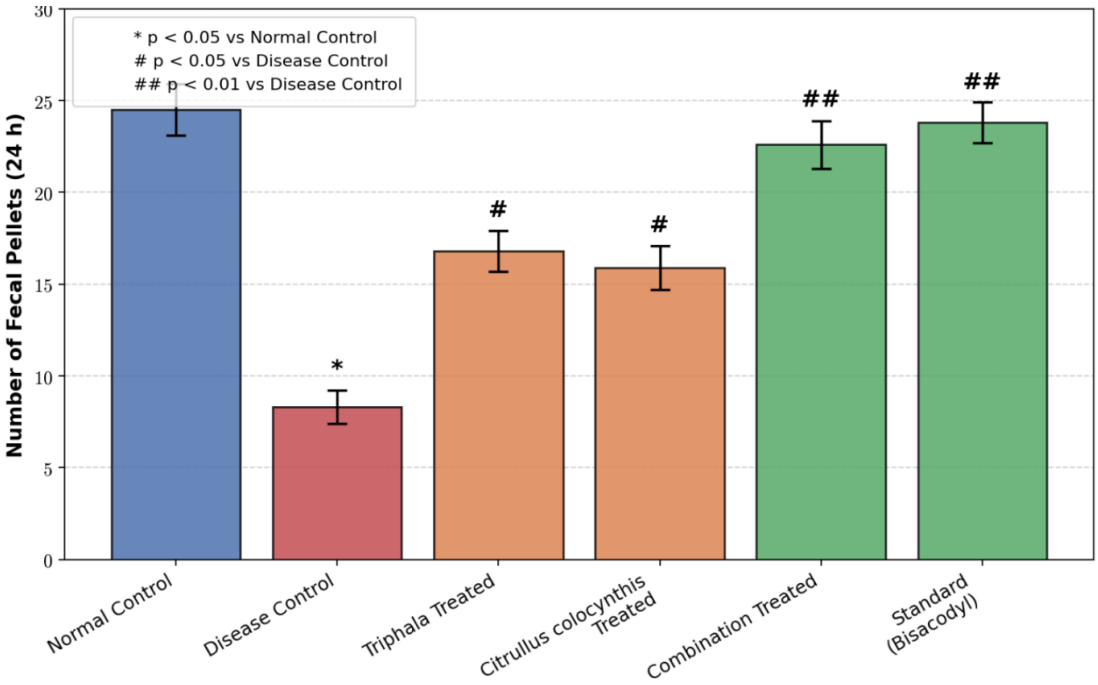

Figure 3: Effect of Different Treatments on Fecal Output.

4.4 Effect on Fecal Water Content

The disease control group showed a significant decrease in fecal water content due to reduced intestinal secretion caused by loperamide. Administration of Triphala churna and *Citrullus colocynthis* churna individually improved fecal moisture content, while the combination-treated group demonstrated maximum fecal water content comparable to the standard drug-treated group.

Table 4: Effect of Treatments on Fecal Water Content.

Groups	Fecal Water Content (%)
Normal Control	58.4 ± 1.8
Disease Control	29.7 ± 1.2*
Triphala Treated	45.3 ± 1.6#
***Citrullus colocynthis* Treated**	43.8 ± 1.4#
Combination Treated	55.9 ± 1.7##
Standard (Bisacodyl)	57.1 ± 1.5##

Values expressed as Mean ± SEM (n = 6). *Significant compared to normal control ($p < 0.05$). #Significant compared to disease control ($p < 0.05$). ##Highly significant compared to disease control ($p < 0.01$).

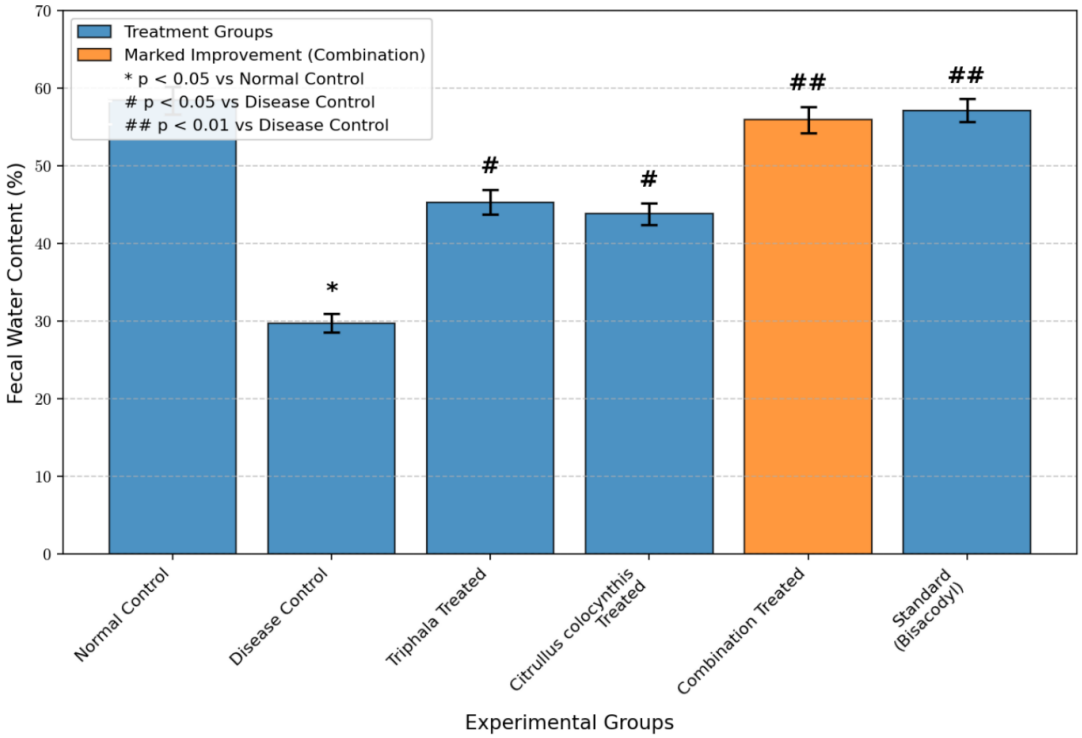

Figure 4: Effect of Treatments on Fecal Water Content.

4.5 Comparative Effect with Standard Drug

The combination of Triphala churna and *Citrullus colocynthis* demonstrated laxative activity comparable to the standard drug Bisacodyl. Significant improvement in fecal output and fecal water content was observed in the combination-treated group compared to disease control animals. The findings suggest a synergistic interaction between both herbal formulations in improving gastrointestinal motility and stool consistency.

5. DISCUSSION

Constipation is commonly associated with decreased intestinal motility, reduced water secretion, and prolonged fecal retention in the colon. In the present study, constipation was induced using loperamide hydrochloride, an opioid receptor agonist known to suppress gastrointestinal propulsion by inhibiting acetylcholine release in the enteric nervous system. Loperamide also increases water absorption from the intestinal lumen, resulting in dry and hard feces with reduced bowel movement frequency. The disease control group exhibited a marked reduction in fecal output and fecal water content, confirming successful induction of constipation in experimental animals.

Treatment with Triphala churna demonstrated a significant improvement in fecal output and fecal moisture content. The laxative effect of Triphala may be attributed to the presence of

phytoconstituents such as gallic acid, tannins, flavonoids, chebulagic acid, and vitamin C. These bioactive compounds are reported to stimulate gastrointestinal motility, improve intestinal secretion, and promote bowel evacuation. In Ayurveda, Triphala is traditionally used as a digestive tonic and mild natural laxative that helps maintain normal bowel function without causing dependency. The antioxidant and anti-inflammatory properties of Triphala may additionally contribute to improved gastrointestinal health and intestinal transit.

Citrullus colocynthis also showed notable laxative activity in loperamide-induced constipated rats. The pharmacological activity of the plant may be associated with the presence of alkaloids, glycosides, terpenoids, flavonoids, and resins. These constituents are known to stimulate intestinal smooth muscle contraction and enhance gastrointestinal secretion, thereby facilitating bowel movement and stool softening. Previous pharmacological reports have also demonstrated that *Citrullus colocynthis* possesses digestive, anti-inflammatory, antioxidant, and gastroprotective activities, supporting its traditional medicinal use in gastrointestinal disorders.

The combination-treated group exhibited greater laxative activity compared to the individual treatment groups, indicating a possible synergistic interaction between Triphala and *Citrullus colocynthis*. The synergistic effect may be due to the combined action of multiple phytoconstituents acting through different mechanisms to improve intestinal motility and fecal hydration. Triphala may primarily enhance bowel movement and antioxidant protection, while *Citrullus colocynthis* may increase intestinal secretion and smooth muscle stimulation. Together, these mechanisms could have contributed to enhanced fecal output and increased fecal water content observed in the present study.

Improvement in intestinal motility and water retention was evident from the significant increase in fecal pellet count and fecal moisture content in the combination-treated group. Increased fecal water content indicates enhanced intestinal secretion and reduced water absorption from the colon, leading to softer stools and easier defecation. Restoration of body weight in treated animals further suggests improvement in gastrointestinal function and general physiological condition following treatment.

The findings of the present study are in agreement with previous reports demonstrating the laxative activity of Triphala and medicinal plants used in traditional systems of medicine. Earlier studies have reported that Triphala improves bowel movements and gastrointestinal

transit with minimal adverse effects. Similarly, studies on herbal laxatives and *Citrullus colocynthis* have shown enhancement of intestinal motility and fecal water accumulation in experimentally induced constipation models. The current study extends these findings by demonstrating the synergistic laxative potential of the combined polyherbal formulation.

6. CONCLUSION

The present study demonstrated that the combination of Triphala and *Citrullus colocynthis* possesses significant synergistic laxative activity in loperamide-induced constipated Wistar rats. The polyherbal combination significantly improved fecal output, fecal water content, and intestinal motility compared to disease control animals. The combination-treated group exhibited activity comparable to the standard drug Bisacodyl without producing observable toxicity or adverse behavioral changes. The findings suggest that the synergistic interaction of phytoconstituents present in Triphala and *Citrullus colocynthis* may provide an effective and safer herbal alternative for the management of constipation.

7. Future Scope

Further studies are required to scientifically validate and optimize the therapeutic potential of the polyherbal formulation. Detailed phytochemical standardization should be performed to identify and quantify the active constituents responsible for laxative activity. Histopathological studies of gastrointestinal tissues may provide better understanding regarding the protective and functional effects of the formulation on intestinal architecture. Chronic toxicity studies are necessary to evaluate the long-term safety profile of the formulation during prolonged administration. Clinical evaluation in human subjects is essential to establish efficacy, safety, and dosage standardization for therapeutic application. In addition, development of a standardized herbal laxative formulation with improved stability, palatability, and patient compliance may enhance its potential for future pharmaceutical use.

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