Jurnal Jamu Indonesia (2026) 11(6): 518–524
https://jamu-journal.ipb.ac.id/index.php/JJI/index

ISSN 2407-7178
eISSN 2407-7763

Research Article
DOI: https://doi.org/10.29244/jji.v11i6.500

Integrated Laxative Effects of *Plantago major* Leaf Extract on Intestinal Function and Colonic Histology in Loperamide-Induced Constipated Rats

Efek Laksatif Ekstrak Daun *Plantago major* terhadap Fungsi Usus dan Histologi Kolon pada Tikus Putih yang Diinduksi Loperamide

Siti Sadiah[1,2], Amaq Fadholly[1]*, Isdoni Isdoni[3], I Ketut Mudite Adnyane[4], Alifia Marcelyaji Nanginda[5], Nabila Andani[5]

[1]*Division of Pharmacology and Toxicology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia*
[2]*Tropical Biopharmaca Research Center, IPB University, Bogor 16128, Indonesia*
[3]*Division of Physiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia*
[4]*Division of Anatomy, Histology and Embryology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia*
[5]*Undergraduate Program of Veterinary Medicine, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia*

ARTICLE INFO

Article history:
Received on: 2026-03-16
Revised on: 2026-09-30
Accepted on: 2026-09-30
Published on: 2026-11-02

Keywords:
Bisacodyl
Constipation
Goblet cell
Intestinal transit
Mucin

Kata kunci:
Bisakodil
Konstipasi
Musin
Sel goblet
Transit usus

ABSTRACT

This study evaluated the laxative activity of *Plantago major* leaf extract in loperamide-induced constipated rats. Thirty male Sprague-Dawley rats received no treatment, loperamide alone, loperamide with bisacodyl, or loperamide with *P. major* extract at 50, 100, or 200 mg/kg body weight (BW). Loperamide reduced fecal number, weight, and water content, intestinal transit ratio, muscular thickness, goblet cell number, and goblet cell area ($p < 0.05$). The extract increased fecal number and weight, improved intestinal transit, and partially restored colonic histology; 100 mg/kg BW was the only dose that restored both muscular thickness and goblet cell area. *P. major* leaf extract therefore has laxative activity and is a candidate natural agent for the management of constipation.

ABSTRAK

Penelitian ini mengevaluasi aktivitas laksatif ekstrak daun *Plantago major* pada tikus yang diinduksi konstipasi dengan loperamid. Tiga puluh ekor tikus jantan Sprague-Dawley tidak diberi perlakuan, diberi loperamid saja, loperamid dengan bisakodil, atau loperamid dengan ekstrak *P. major* dosis 50, 100, atau 200 mg/kg bobot badan (BB). Loperamid menurunkan jumlah, berat, dan kadar air feses, rasio transit usus, ketebalan otot, serta jumlah dan luas sel goblet ($p < 0,05$). Ekstrak meningkatkan jumlah dan berat feses, memperbaiki transit usus, dan memulihkan sebagian histologi kolon; dosis 100 mg/kg BB merupakan satu-satunya dosis yang memulihkan ketebalan otot sekaligus luas sel goblet. Ekstrak daun *P. major* memiliki aktivitas laksatif dan berpotensi dikembangkan sebagai agen alami untuk penanganan konstipasi.

**Corresponding author:*
Amaq Fadholly (amaqfadholly@apps.ipb.ac.id)

Citation: Sadiah, S., Fadholly, A., Isdoni, I., Adnyane, I. K. M., Nanginda, A. M., & Andani, N. (2026). Integrated laxative effects of *Plantago major* leaf extract on intestinal function and colonic histology in loperamide-induced constipated rats. *Jurnal Jamu Indonesia*, *11*(6), 518–524. https://doi.org/10.29244/jji.v11i6.500

© 2026 Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (http://creativecommons.org/licenses/by-nc-sa/4.0/)

1. INTRODUCTION

Constipation is a prevalent gastrointestinal disorder affecting individuals across all age groups, with increasing incidence among older populations. Clinically, constipation is characterized by reduced bowel movement frequency, excessive straining, hardened stools, and sensations of incomplete evacuation. Chronic constipation impairs health-related quality of life and work productivity (Tomita et al., 2021) and may also predispose individuals to complications such as hemorrhoids, diverticular disease, and colorectal disorders (Heidelbaugh et al., 2021). Pharmacological management of constipation commonly involves osmotic, stimulant, or bulk-forming laxatives. Although these agents help many patients, a large proportion remain dissatisfied with them because of inadequate symptom relief and side effects such as diarrhea (Harris et al., 2017; Hayashi et al., 2022). Consequently, there is growing interest in plant-based laxatives, several of which have improved stool output, intestinal transit, and colonic histology in loperamide-induced constipation models (Hajji et al., 2020; Lee et al., 2020).

Plantago major L., a member of the Plantaginaceae family, is widely distributed and has traditionally been used for various gastrointestinal disorders, including constipation (Karami et al., 2020; Najafian et al., 2018). The leaves contain various bioactive constituents, including polysaccharides, flavonoids, iridoid glycosides, phenolic acids, and other secondary metabolites that may contribute to the diverse biological activities of *P. major* (Adom et al., 2017; Bouali et al., 2024; Sari et al., 2024). Recent experimental evidence involving *P. major*-containing preparations has also demonstrated improvements in fecal parameters and gastrointestinal transit in loperamide-induced constipation models (Fadholly et al., 2026). However, constipation is not solely a functional disorder. Experimental loperamide-induced constipation has also been associated with structural and secretory alterations of the colon, including changes in mucosal and muscular thickness and reduced colonic mucus production (Shimotoyodome et al., 2000). Colonic mucus, produced primarily by goblet cells, plays a critical role in lubrication, epithelial protection, and facilitation of fecal passage. Alterations in intestinal mucus and goblet cell function may also influence intestinal transit and epithelial homeostasis (Fang et al., 2023). Therefore, evaluating both functional outcomes and histological changes is essential for understanding the full therapeutic potential of laxative agents.

Evidence for *P. major* itself is still indirect. The only laxative study involving the plant tested its leaf extract in combination with *Cassia alata* and measured stool output and transit, not colonic structure (Fadholly et al., 2026), so the contribution of *P. major* alone and its effect on the colonic wall remain unknown. This study therefore evaluated *P. major* leaf extract at three doses in loperamide-induced constipated rats, combining stool parameters and intestinal transit with colonic histomorphometry and goblet cell staining, to determine whether functional and structural recovery occur together.

2. METHODS

2.1. Plant Material, Chemicals, and Extraction

Fresh leaves of *P. major* were collected from the Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia (Reference number: BMK0205092016). Plant materials were authenticated at the center prior to use. The leaves were washed with distilled water, oven-dried at 40 °C until constant weight, and ground into a fine powder using a laboratory grinder. Extraction was performed by maceration using 70% ethanol at a ratio of 1:10 (w/v). The powdered leaves were soaked in solvent for 24 h at room temperature (25 ± 2 °C) with intermittent stirring every 6 h. The extraction process was repeated three consecutive times using fresh solvent. Combined filtrates were concentrated under reduced pressure using a rotary evaporator (R-300, Büchi Labortechnik, Flawil, Switzerland) at 40 °C and subsequently dried in a laboratory oven at 40 °C until a constant dry extract was obtained. The extraction yield was 10.0% (w/w), calculated from 30 g of dried extract obtained from 300 g of dried *P. major* leaf powder. Loperamide hydrochloride (Sigma-Aldrich, St. Louis, MO, USA), bisacodyl (Dulcolax®, 5 mg; Boehringer Ingelheim, Jakarta, Indonesia), activated charcoal (Samiraschem, Jakarta, Indonesia), gum acacia (Samiraschem, Jakarta, Indonesia), hematoxylin–eosin staining reagents (Merck, Darmstadt, Germany), alcian blue pH 2.5 (Merck, Darmstadt, Germany), periodic acid–Schiff (PAS) reagents (Sigma-Aldrich, St. Louis, MO, USA), and neutral buffered formalin (Merck, Darmstadt, Germany) were used for the experimental procedures.

2.2. Experimental Animals

Thirty male Sprague-Dawley rats aged 6–8 weeks and weighing 150–200 g were obtained from the Laboratory Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia. Animals were housed in polypropylene cages (five rats per cage) under controlled laboratory conditions (22 ± 2 °C, 50–60% relative humidity, and a 12 h light/dark cycle) with free access to standard pellet feed and drinking water ad libitum. Animals were acclimatized for seven days before the experiment. These procedures were approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University (Reference number: 154/KEH/SKE/XII/2023).

2.3. Experimental Design

The experimental design consisted of several parts including preparation and extraction of *P. major* leaves, animal acclimatization, induction of constipation using loperamide, administration of treatments, evaluation of fecal parameters, intestinal transit assessment, tissue collection and histological examination, and statistical analysis. Animals were randomly assigned into six groups (n = 5/group). The individual rat was

considered the experimental unit for all parameters except water intake, which was measured per cage. The groups were P0: Normal control (without loperamide induction and without treatment), P1: Negative control (loperamide 5 mg/kg body weight [BW]), P2: Positive control (loperamide 5 mg/kg BW + bisacodyl 0.5 mg/kg BW), P3: Loperamide 5 mg/kg BW + *P. major* extract 50 mg/kg BW, P4: Loperamide 5 mg/kg BW + *P. major* extract 100 mg/kg BW, P5: Loperamide 5 mg/kg BW + *P. major* extract 200 mg/kg BW. Constipation was induced by oral administration of loperamide hydrochloride at a dose of 5 mg/kg BW once daily for three consecutive days, except in the normal control group (P0). On day 4, the respective treatments were administered orally by gastric gavage according to the assigned groups: bisacodyl at 0.5 mg/kg BW for P2 and *P. major* leaf extract at 50, 100, and 200 mg/kg BW for P3, P4, and P5, respectively. The negative control group (P1) received loperamide induction without subsequent treatment.

2.4. Evaluation of Fecal Parameters

Feces were collected individually over a 24 h period after treatment administration. The total number of fecal pellets was counted manually. Wet fecal weight was measured using an analytical balance (Pioneer PX, Ohaus, Parsippany, NJ, USA). Samples were subsequently dried at 60 °C until constant weight to obtain dry fecal weight. Fecal water content was calculated from the wet fecal weight (W_{wet}) and the dry fecal weight (W_{dry}) as follows:

$$\text{Fecal water content (\%)} = (W_{wet} - W_{dry})/W_{wet} \times 100 \quad (1)$$

2.5. Intestinal Transit Test

Following the fecal collection period, rats were orally administered an activated charcoal suspension consisting of 10% activated charcoal in 5% gum acacia at a dose volume of 1 mL/100 g BW. Thirty minutes after charcoal administration, animals were euthanized using ketamine (100 mg/kg BW) and xylazine (20 mg/kg BW), administered intraperitoneally. The entire small intestine from the pylorus to the ileocecal junction was carefully excised and gently laid out and extended without stretching the tissue. The total intestinal length and the distance traveled by the charcoal marker were measured incrementally using a digital caliper. Intestinal transit ratio was calculated from the distance traveled by the charcoal marker (D) and the total length of the small intestine (L) as follows:

$$\text{Intestinal transit ratio (\%)} = D/L \times 100 \quad (2)$$

2.6. Histological Analysis

Representative segments of the colon were removed for histological examination. The tissue was fixed for 24 h in 10% neutral buffered formalin, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were cut at 5 µm with a rotary microtome (Leica RM2235, Wetzlar, Germany). General histology was assessed using standard histology protocols with hematoxylin–eosin (HE) stained tissue sections, alcian blue (AB, pH 2.5) stained tissue sections to visualize acidic mucins, and periodic acid–Schiff (PAS) stained tissue sections to detect neutral mucins. In each animal, 10 microscopic fields were examined in a section of colon. Light microscopic images were captured with a BX53 light microscope (Olympus, Tokyo, Japan) equipped with a digital camera at 100× and 400× magnification, depending on the histological parameters. Quantification was done using ImageJ version 1.54 (National Institutes of Health, Bethesda, MD, USA). The region of AB-stained goblet cells was evaluated from AB-stained sections by measuring the region of AB-positive goblet cell stain in relation to the entire tissue area in each chosen microscopic field. The mean value of the 10 microscopic fields for each animal was calculated and used as representative values for statistical analysis. Histological assessment was not performed in a blinded manner to treatment groups.

2.7. Statistical Analysis

Data are presented as mean ± standard deviation (SD), which describes variability among biological replicates. For parameters measured individually, the individual rat was considered the experimental unit (n = 5/group). Data were assessed for normality using the Shapiro–Wilk test and homogeneity using Levene's test, followed by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post hoc test. Water intake was measured per cage and was therefore reported descriptively rather than subjected to inferential statistical analysis. Differences were considered significant at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Effects of *P. major* Extract on Fecal Parameters

Loperamide lowered fecal number ($F(5,24) = 31.24$), fecal weight ($F(5,24) = 7.82$) and fecal water content ($F(5,24) = 21.76$; all $p < 0.001$) compared with the normal control (**Table 1**). All three extract doses increased fecal number and fecal weight above the negative control ($p < 0.05$); fecal weight in every treated group returned to the level of the normal control, and at 100 mg/kg BW (P4) fecal number did not differ from it either. Food intake was lower in the negative control than in all other groups ($F(5,24) = 4.44$, $p = 0.005$), whereas water intake, measured per cage, is reported descriptively.

Loperamide slows propulsion and enhances net fluid absorption in the gut, which yields fewer, smaller and drier pellets (Sandhu et al., 1981; Shimotoyodome et al., 2000). The recovery of pellet number and weight in all extract-treated groups therefore points to restored propulsive activity. Stool hydration behaved differently: fecal water content rose above the negative control only at 50 mg/kg BW (P3), did not differ from it at 100 mg/kg BW, and was lowest at 200 mg/kg BW (**Table 1**). Output and hydration thus appear to respond through partly separate mechanisms, a point taken up again in Section 3.4.

Table 1. Food and water intake, fecal parameters and intestinal transit ratio of loperamide-induced constipated rats treated with *P. major* leaf extract

Group	Food intake (g)	Fecal number (pellets)	Fecal weight (g)	Fecal water content (%)	Water intake (mL)	Intestinal transit ratio (%)
P0	19.73 ± 2.50^{a}	68.60 ± 1.67^{a}	13.60 ± 0.80^{a}	56.60 ± 0.29^{ab}	31.25 ± 2.35	84.20 ± 3.45^{a}
P1	13.71 ± 1.25^{b}	34.40 ± 3.36^{c}	8.14 ± 0.46^{b}	52.41 ± 1.93^{cd}	28.75 ± 6.10	48.16 ± 9.31^{c}
P2	19.75 ± 3.37^{a}	58.40 ± 6.07^{b}	12.87 ± 0.74^{a}	58.70 ± 0.25^{a}	36.25 ± 5.62	68.63 ± 4.46^{b}
P3	19.38 ± 3.75^{a}	58.40 ± 5.13^{b}	11.60 ± 2.12^{a}	55.70 ± 3.10^{ab}	27.50 ± 6.78	64.05 ± 2.20^{b}
P4	19.60 ± 1.17^{a}	61.60 ± 5.59^{ab}	12.87 ± 2.07^{a}	54.53 ± 0.80^{bc}	31.25 ± 7.70	71.21 ± 3.62^{b}
P5	20.00 ± 2.45^{a}	54.40 ± 4.51^{b}	11.57 ± 2.10^{a}	49.60 ± 0.41^{d}	25.00 ± 0.00	68.97 ± 3.23^{b}
p-value	0.005	< 0.001	< 0.001	< 0.001	–	< 0.001

Note. Values are mean ± SD (n = 5 rats per group) for individually measured parameters. P0 = normal control; P1 = negative control (loperamide 5 mg/kg BW); P2 = positive control (loperamide 5 mg/kg BW + bisacodyl 0.5 mg/kg BW); P3, P4 and P5 = loperamide 5 mg/kg BW + *P. major* leaf extract at 50, 100 and 200 mg/kg BW, respectively. Different superscript letters within a column indicate significant differences between groups (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$), with a assigned to the highest mean; p-values are from one-way ANOVA. Water intake was measured per cage and is presented descriptively; therefore, no statistical comparison was performed. BW, body weight; HSD, honestly significant difference; SD, standard deviation.

3.2. Effects on Intestinal Transit

Loperamide reduced the charcoal transit ratio from 84.20% to 48.16% ($F(5,24) = 27.87$, $p < 0.001$; **Table 1**). All three extract doses raised it to 64.05–71.21%, values that did not differ from bisacodyl (68.63%) but remained below the normal control. Gains in small-intestinal propulsion of this size have been reported for other plant preparations, probiotics and soluble fibres in loperamide models (Li et al., 2023; Qiu et al., 2022; Zhang et al., 2025). The flavonoid quercetin, for example, restored motility in loperamide-treated rats through muscarinic M2/M3 receptor signalling (Kim et al., 2018); whether the flavonoids and phenylethanoid glycosides of *P. major* leaves (Bouali et al., 2024) act through the same pathway has not yet been tested.

3.3. Effects on Colonic Histomorphology

Loperamide thinned the muscular layer ($F(5,24) = 31.32$, $p < 0.001$) and reduced both the number of AB-positive goblet cells ($F(5,24) = 13.73$, $p < 0.001$) and goblet cell area ($F(5,24) = 8.43$, $p < 0.001$), whereas mucosal thickness did not differ among groups ($F(5,24) = 0.91$, $p = 0.49$; **Table 2**). Muscular thickness in P4 and P5 returned to the level of the normal control, as it did with bisacodyl, but remained low at 50 mg/kg BW (P3).

The recovery of muscular thickness at 100 and 200 mg/kg BW parallels the improvement in transit at these doses and suggests that the extract acted on the muscle layer that drives propulsion. At 50 mg/kg BW, however, transit improved while the muscular layer stayed thin, so the functional effect at this dose cannot rest on structural recovery of the muscle alone. The unchanged mucosal thickness indicates that the three-day loperamide course affected the muscular and secretory compartments rather than overall mucosal architecture, and that the extract acted selectively on these compartments (Gallego-Barceló et al., 2024).

Table 2. Colonic histomorphometry of loperamide-induced constipated rats treated with *P. major* leaf extract

Group	Muscular thickness (µm)	Mucosal thickness (µm)	AB-positive goblet cells (cells/field, 400×)	Goblet cell area (%)
P0	250.17 ± 27.04^{a}	412.38 ± 36.01	251.80 ± 49.23^{b}	23.61 ± 5.76^{a}
P1	131.24 ± 25.31^{b}	395.30 ± 44.87	154.60 ± 37.82^{c}	14.04 ± 2.28^{b}
P2	212.27 ± 22.85^{a}	444.31 ± 59.81	302.40 ± 42.95^{ab}	14.22 ± 2.32^{b}
P3	120.76 ± 11.18^{b}	382.70 ± 53.71	363.40 ± 30.80^{a}	18.73 ± 2.27^{ab}
P4	221.33 ± 22.55^{a}	397.23 ± 58.84	278.80 ± 49.49^{b}	23.25 ± 3.48^{a}
P5	235.06 ± 19.69^{a}	416.28 ± 46.00	237.80 ± 39.42^{b}	16.22 ± 2.11^{b}
p-value	< 0.001	0.492	< 0.001	< 0.001

Note. Values are mean ± SD (n = 5 rats per group; mean of 10 microscopic fields per rat). P0 = normal control; P1 = negative control (loperamide 5 mg/kg BW); P2 = positive control (loperamide 5 mg/kg BW + bisacodyl 0.5 mg/kg BW); P3, P4 and P5 = loperamide 5 mg/kg BW + *P. major* leaf extract at 50, 100 and 200 mg/kg BW, respectively. Different superscript letters within a column indicate significant differences between groups (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$), with a assigned to the highest mean; p-values are from one-way ANOVA. Mucosal thickness did not differ among groups and carries no letters. AB, alcian blue; BW, body weight; HSD, honestly significant difference; SD, standard deviation.

3.4. Effects on Goblet Cells and Mucin Production

Alcian blue staining showed that goblet cell number and goblet cell area did not move together (**Table 2** and **Figure 1**). Bisacodyl raised goblet cell number to at least the normal level (302.40 ± 42.95 versus 251.80 ± 49.23 cells) and the 50 mg/kg BW dose raised it above that level (363.40 ± 30.80 cells), yet goblet cell area in the bisacodyl group stayed at the level of the negative control, and only the 100 mg/kg BW dose restored both measures. Many small AB-positive profiles may reflect mucin being discharged faster than it is replenished, whereas a normal area suggests that the cells have refilled their granules. Goblet cells secrete the mucus that lubricates the colonic lumen and protects the epithelium (Nyström et al., 2021), and loperamide lowers colonic mucus in rats (Shimotoyodome et al., 2000), so recovery of goblet cell area is the more telling sign of restored secretory capacity. Bisacodyl acts largely on motility, increasing interstitial cells of Cajal and c-Kit expression in constipated rats (Wang et al., 2018), and would therefore be expected to restore function without necessarily refilling goblet cells, as observed here.

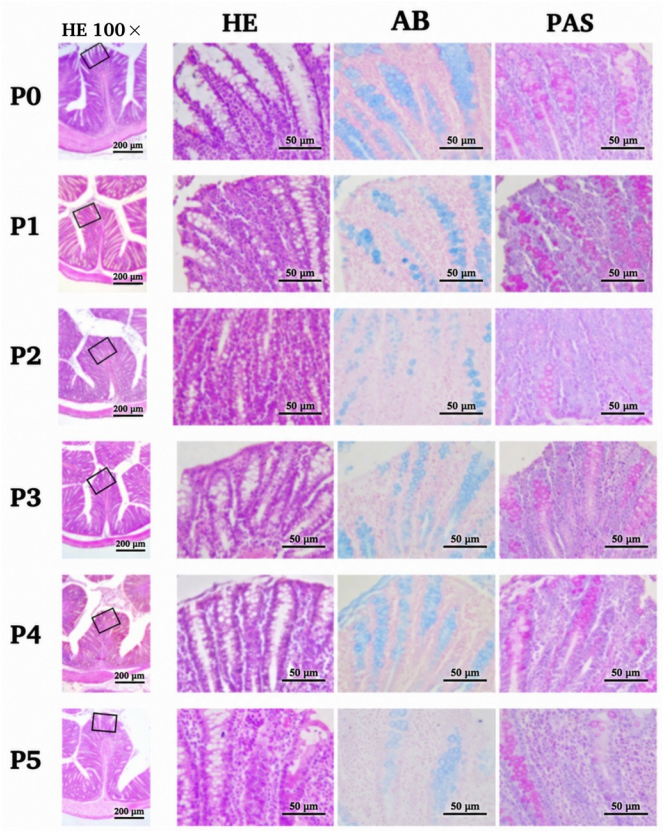


Figure 1. Colonic histology of the six groups stained with hematoxylin–eosin (HE), alcian blue pH 2.5 (AB) and periodic acid–Schiff (PAS). The left column shows overview sections at 100× (scale bar 200 μm); the boxes mark the region shown at 400× in the HE, AB and PAS columns (scale bar 50 μm). P0, normal control; P1, negative control (loperamide 5 mg/kg BW); P2, positive control (bisacodyl 0.5 mg/kg BW); P3–P5, *P. major* leaf extract at 50, 100 and 200 mg/kg BW.

The dose–response was not monotonic. The 200 mg/kg BW dose restored muscular thickness and transit as well as 100 mg/kg BW did, but gave the lowest fecal water content and a goblet cell area no better than the negative control. Together with the bisacodyl group, which recovered function without recovering goblet cell area, these observations suggest that functional recovery of motility and restoration of colonic histology do not necessarily proceed in parallel (Onica et al., 2025; Yang et al., 2026).

Candidate constituents can be proposed, although not yet assigned. *P. major* leaves contain the phenylethanoid glycoside acteoside (verbascoside) and the iridoid aucubin (Bouali et al., 2024; Sari et al., 2024); verbascoside increased goblet cell number in injured rat colon (Değer et al., 2021), and quercetin enhanced both motility and mucin secretion in loperamide-treated rats (Kim et al., 2018). Leaf polysaccharides may add a bulk-forming contribution (Adom et al., 2017). Because the extract in this study was not standardized to marker compounds, these links remain hypotheses; quantifying aucubin and acteoside in the extract and measuring mucin 2 (MUC2) expression and muscarinic signalling in the colon would allow them to be tested directly.

4. CONCLUSION

P. major leaf extract relieved loperamide-induced constipation in rats. All three doses increased fecal number and weight and restored small-intestinal transit to the level achieved with bisacodyl, while the colonic response depended on dose: muscular thickness recovered at 100 and 200 mg/kg BW, and goblet cell area recovered only at 100 mg/kg BW. The 100 mg/kg BW dose gave the most balanced response, being the only dose that restored both muscular thickness and goblet cell area while improving stool output and transit, and it is the dose best suited to follow-up work on the active constituents and mechanism of the extract.

AUTHOR CONTRIBUTIONS

Conceptualization, S.S., A.F. and I.; methodology, A.F., I. and I.K.M.A.; validation, S.S., A.F. and I.; formal analysis, I.K.M.A., A.M.N. and N.A.; investigation, A.M.N. and N.A.; resources, A.F. and I.K.M.A.; data curation, S.S. and I.; writing—original draft preparation, S.S., A.F., A.M.N. and N.A.; writing—review and editing, I. and I.K.M.A.; visualization, I.K.M.A., A.M.N. and N.A.; supervision, S.S. and A.F.; project administration, I.K.M.A.; funding acquisition, S.S. and I. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

All animal study procedures were approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia (Reference number: 154/KEH/SKE/XII/2023).

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data supporting this study are available on reasonable request from the corresponding author.

FUNDING

This study was funded by Riset Fundamental, IPB University with reference number 422/IT.3.D10/PT.01.03/P/B/2023.

ACKNOWLEDGMENT

The authors acknowledge the Laboratory of Pharmacology and Toxicology, Laboratory of Histology, Laboratory Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, also Tropical Biopharmaca Research Centre, IPB University for providing laboratory facilities and technical support.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors thoroughly reviewed, edited, and verified the entire content to ensure that it accurately represents their own ideas, data, analyses, and interpretations. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

REFERENCES

Adom, M. B., Taher, M., Mutalabisin, M. F., Amri, M. S., Kudos, M. B. A., Sulaiman, M. W. A. W., Sengupta, P., & Susanti, D. (2017). Chemical constituents and medical benefits of *Plantago major*. *Biomedicine & Pharmacotherapy, 96*, 348–360. https://doi.org/10.1016/j.biopha.2017.09.152

Bouali, A., Spissu, Y., Barberis, A., Fadda, A., Azara, E., Orrù, G., Scano, A., Palmieri, G., D'hallewin, G., & El Ferchichi Ouarda, H. (2024). Phytochemical evaluation and exploration of some biological activities of aqueous and ethanolic extracts of two species of the genus *Plantago* L. *PLOS ONE, 19*(2), e0298518. https://doi.org/10.1371/journal.pone.0298518

Değer, A. N., Özyiğit, F., Koçak, F. E., Bayhan, Z., Zeren, S., Arık, Ö., & Değer, H. (2021). Corrective effect of verbascoside on histomorphological differences and oxidative stress in colon mucosa of rats in which colon ischemia-reperfusion injury was induced. *Turkish Journal of Gastroenterology, 32*(7), 548–559. https://doi.org/10.5152/tjg.2021.19747

Fadholly, A., Sadiah, S., Heryanto, R., & Ramadhan, M. K. (2026). The laxative efficacy of a mixed extract of *Cassia alata* with *Plantago major* in rats. *Open Veterinary Journal, 16*(4), 2313–2319. https://doi.org/10.5455/OVJ.2026.v16.i4.31

Fang, F., Liu, Y., Xiong, Y., Li, X., Li, G., Jiang, Y., Hou, X., & Song, J. (2023). Slowed intestinal transit induced by less mucus in intestinal goblet cell Piezo1-deficient mice through impaired epithelial homeostasis. *International Journal of Molecular Sciences, 24*(18), 14377. https://doi.org/10.3390/ijms241814377

Gallego-Barceló, P., Benítez-Álvarez, D., Bagues, A., Silván-Ros, B., Montalbán-Rodríguez, A., López-Gómez, L., Vera, G., del Castillo, M. D., Uranga, J. A., & Abalo, R. (2024). *Ex vivo* study of colon health, contractility and innervation in male and female rats after regular exposure to instant cascara beverage. *Foods, 13*(16), 2474. https://doi.org/10.3390/foods13162474

Hajji, N., Wannes, D., Jabri, M.-A., Rtibi, K., Tounsi, H., Abdellaoui, A., & Sebai, H. (2020). Purgative/laxative actions of *Globularia alypum* aqueous extract on gastrointestinal-physiological function and against loperamide-induced constipation coupled to oxidative stress and inflammation in rats. *Neurogastroenterology & Motility, 32*(8), e13858. https://doi.org/10.1111/nmo.13858

Harris, L. A., Horn, J., Kissous-Hunt, M., Magnus, L., & Quigley, E. M. M. (2017). The Better Understanding and Recognition of the Disconnects, Experiences, and Needs of Patients with Chronic Idiopathic Constipation (BURDEN-CIC) study: Results of an online questionnaire. *Advances in Therapy, 34*(12), 2661–2673. https://doi.org/10.1007/s12325-017-0633-5

Hayashi, T., & Miwa, H. (2022). Treatment satisfaction with current medications for chronic constipation. *Journal of Clinical Gastroenterology, 56*(1), e64–e70. https://doi.org/10.1097/MCG.0000000000001473

Heidelbaugh, J., Martinez de Andino, N., Pineles, D., & Poppers, D. M. (2021). Diagnosing constipation spectrum disorders in a primary care setting. *Journal of Clinical Medicine, 10*(5), 1092. https://doi.org/10.3390/jcm10051092

Karami, N., Karimi, M., & Bahmani, M. (2020). Constipation: An ethno-botanical study of medicinal plants used for constipation in Shahrekord city, Chaharmahal & Bakhtiari province, Iran. *Plant Biotechnology Persa, 2*(1), 1–7. https://doi.org/10.29252/pbp.2.1.1

Kim, J. E., Lee, M. R., Park, J. J., Choi, J. Y., Song, B. R., Son, H. J., Choi, Y. W., Kim, K. M., Hong, J. T., & Hwang, D. Y. (2018). Quercetin promotes gastrointestinal motility and mucin secretion in loperamide-induced constipation of SD rats through regulation of the mAChRs downstream signal. *Pharmaceutical Biology, 56*(1), 309–317. https://doi.org/10.1080/13880209.2018.1474932

Lee, H.-J., Choi, E. J., Park, S., & Lee, J.-J. (2020). Laxative and antioxidant effects of ramie (*Boehmeria nivea* L.) leaf extract in experimental constipated rats. *Food Science & Nutrition, 8*(7), 3389–3401. https://doi.org/10.1002/fsn3.1619

Li, Y., Wang, A., Nie, M., Chen, Z., Wang, L., Liu, L., Wang, F., & Tong, L. (2023). Barley vinegar relieves loperamide-induced constipation in mice via the modulation of the gut microbiota and serum metabolism. *Fermentation, 9*(11), 962. https://doi.org/10.3390/fermentation9110962

Najafian, Y., Hamedi, S. S., Kaboli Farshchi, M., & Feyzabadi, Z. (2018). *Plantago major* in traditional Persian medicine and modern phytotherapy: A narrative review. *Electronic Physician, 10*(2), 6390–6399. https://doi.org/10.19082/6390

Nyström, E. E. L., Martinez-Abad, B., Arike, L., Birchenough, G. M. H., Nonnecke, E. B., Castillo, P. A., Svensson, F., Bevins, C. L., Hansson, G. C., & Johansson, M. E. V. (2021). An intercrypt

subpopulation of goblet cells is essential for colonic mucus barrier function. *Science, 372*(6539), eabb1590. https://doi.org/10.1126/science.abb1590

Onica, E.-C., Dumitru, C.-S., Zara, F., Raica, M., Suciu, C. S., Barb, A. C., Ene, O.-A., Tarta, C., & Novacescu, D. (2025). Pentachroma O-H: A five-color histological staining method for enhanced intestinal tissue analysis. *International Journal of Molecular Sciences, 26*(22), 10811. https://doi.org/10.3390/ijms262210811

Qiu, B., Zhu, L., Zhang, S., Han, S., Fei, Y., Ba, F., Berglund, B., Li, L., & Yao, M. (2022). Prevention of loperamide-induced constipation in mice and alteration of 5-hydroxytryotamine signaling by *Ligilactobacillus salivarius* Li01. *Nutrients, 14*(19), 4083. https://doi.org/10.3390/nu14194083

Sandhu, B. K., Tripp, J. H., Candy, D. C., & Harries, J. T. (1981). Loperamide: Studies on its mechanism of action. *Gut, 22*(8), 658–662. https://doi.org/10.1136/gut.22.8.658

Sari, K. R. P., Ikawati, Z., Danarti, R., Nafiisah, N. H., & Hertiani, T. (2024). Evaluating the efficacy of ultrasound-assisted extraction of *Plantago major* L. leaves by response surface methodology through determination of aucubin levels. *Journal of Advanced Pharmaceutical Technology & Research, 15*(4), 258–263. https://doi.org/10.4103/japtr.japtr_74_24

Shimotoyodome, A., Meguro, S., Hase, T., Tokimitsu, I., & Sakata, T. (2000). Decreased colonic mucus in rats with loperamide-induced constipation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 126*(2), 203–212. https://doi.org/10.1016/S1095-6433(00)00194-X

Tomita, T., Kazumori, K., Baba, K., Zhao, X., Chen, Y., & Miwa, H. (2021). Impact of chronic constipation on health-related quality of life and work productivity in Japan. *Journal of Gastroenterology and Hepatology, 36*(6), 1529–1537. https://doi.org/10.1111/jgh.15295

Wang, Y.-B., Ling, J., Zhang, W.-Z., Li, G., Qiu, W., Zheng, J.-H., & Zhao, X.-H. (2018). Effect of bisacodyl on rats with slow transit constipation. *Brazilian Journal of Medical and Biological Research, 51*(7), e7372. https://doi.org/10.1590/1414-431X20187372

Yang, F., Wang, G., Huang, M., Liu, X., Tang, S., Li, W., Luo, Y., Bai, J., & Huang, L. (2026). Comparative study of the effectiveness of cellulose, pectin and citrus peel powder in alleviating loperamide-induced constipation. *Foods, 15*(2), 240. https://doi.org/10.3390/foods15020240

Zhang, Z., Liu, B., Liu, W., Liu, X., Zhang, C., Hu, W., & Wu, W. (2025). Different efficacy of five soluble dietary fibers on alleviating loperamide-induced constipation in mice: Influences of different structural features. *International Journal of Molecular Sciences, 26*(3), 1236. https://doi.org/10.3390/ijms26031236

Publisher's Note & Disclaimer

All statements, opinions, and data in this publication were solely the responsibility of the individual authors or contributors and did not necessarily reflect the views of the publisher or editors. The publisher and editors did not guarantee the accuracy, completeness, or reliability of the content, and were not legally responsible for any errors, omissions, or consequences arising from its use. The publisher and editors also disclaimed any liability for injury, damage, or loss to persons or property resulting from the application of ideas, methods, or products mentioned herein. Readers were advised to independently verify all information before relying on it. The publisher accepted no responsibility for any consequences arising from the use of this publication's materials.