Journal of Medicinal Materials, 2026, Vol. 31, No. 1+2 (pp. 113 – 122)

ACUTE ORAL TOXICITY AND LAXATIVE EFFECTS OF *PHARBITIS NIL* (L.) CHOISY SEED EXTRACT

Nguyen Thi Kim Oanh[1,2], Tran Vien Thong[1,3], Truong Huy Hieu[3], Ly Hai Trieu[1,3], Pham Thi Nguyet Hang[1], Nguyen Thi Vinh Hue[4,5], Do Thi Ha[1,*]

[1]National Institute of Medicinal Materials (NIMM), Hanoi 11018, Vietnam;
[2]Thai Binh University of Medicine and Pharmacy, Hung Yen 06122, Vietnam;
[3]Research Center of Ginseng and Medicinal Materials (CGMM), NIMM, Ho Chi Minh City 71016, Vietnam;
[4]Traphaco Joint Stock Company, Hanoi 11719, Vietnam
[5]Faculty of Medicine and Pharmacy, Dainam University, Ha Dong, Ha Noi 12119, Vietnam
*Corresponding author: hado.vdl@nimm.org.vn

Received March 05th, 2026 Accepted March 20th, 2026

Summary

The present study aimed to investigate the acute oral toxicity and evaluate the laxative effect of *Pharbitis nil* (L.) Choisy seed extract in mice. Acute oral toxicity of the extract was assessed in *Swiss albino* mice using the Karber-Behrens method to determine the LD_{50} value. The laxative effect was evaluated in loperamide-induced constipation and normal mice through fecal water content, number and morphology of fecal pellets, intestinal charcoal transit ratio, amount of retained feces in the colon, and gastrointestinal fluid secretion. The results showed that the *P. nil* seed extract exhibited low acute oral toxicity (LD50 = 3.96 g/kg). In the loperamide-induced constipation model, the extract at doses of 150 and 300 mg/kg significantly increased fecal water content compared with the constipation model group, exhibiting effect comparable to the reference drug bisacodyl (1 mg/kg). At a dose of 300 mg/kg, this extract significantly enhanced intestinal motility, comparable to bisacodyl (1 mg/kg), by increasing the intestinal charcoal transit ratio. The extract at doses of 150 and 300 mg/kg also reduced fecal retention in the colon. In normal mice, the extract at both doses, 150 and 300 mg/kg, increased fecal water content and the weight of intestinal contents, indicating stimulation of fluid secretion into the intestinal lumen. In conclusion, the P. *nil* seed extract exhibited significant laxative properties in a loperamide-induced constipation mouse model, providing scientific evidence supporting the use of this medicinal plant in the management of constipation.

Keywords: *Pharbitis nil (L.) Choisy seed extract; Acute oral toxicity; Laxative effect; Constipation; Loperamide.*

1. Introduction

Pharbitis nil (L.) Choisy (*P. nil*), belonging to the family Convolvulaceae, is a plant widely used in traditional medicine in many Asian countries. The seeds of this plant, also known as *Pharbitidis Semen* (Khiên ngưu tử), have been reported to possess purgative, laxative, diuretic, and anti-edematous effects, and are commonly used in herbal medicine for relief and management of constipation and certain gastrointestinal disorders [1],[2]. According to the Vietnamese Pharmacopoeia V, the recommended dose of *P. nil* seeds is 4 – 8 g of crude drug per day in herbal formulations used to support the management of constipation [2].

Phytochemical studies have shown that the *P. nil* seeds contain various bioactive compounds, among which resin glycosides (Pharbitin) are considered the main constituents responsible for the laxative activity of the plant. These compounds are capable of stimulating intestinal motility and increasing fluid secretion into the intestinal lumen, thereby facilitating fecal excretion [3],[4]. In addition, several studies have reported other biological activities of this medicinal plant, including anti-inflammatory, antioxidant, and other pharmacological effects [5],[6].

P. nil seeds have long been used in traditional medicine. To provide further scientific evidence regarding the safety and pharmacological effects of their extract, the present study investigated the acute oral toxicity and laxative activities of *P. nil* seed extract in experimental models. The study results might provide scientific evidence to support the use and development of medicinal products derived from this medicinal material for the management of constipation.

2. Materials and methods

2.1. Materials

The *P. nil* seed extract is derived from our previous report [7]. Briefly, *P. nil* seed powder was extracted with 70% ethanol (1:2, *w/v*) for 48 h at room temperature to get the *P. nil* seed extract. The extract has a moisture content of 11.45% and an extraction yield of 13.18%. The extract was stored at 2-8°C and dissolved in distilled water for dose preparation.

The tested doses of the extract (75, 150, and

300 mg/kg) were selected based on our previous report [7], which were converted from the recommended human herbal dosage according to the monograph on *P. nil* seeds in the Vietnamese Pharmacopoeia V.

2.2. Animals

Swiss albino mice of both sexes (18 – 24 g), collected from the National Institute of Hygiene and Epidemiology, were used to study acute oral toxicity. *ICR* male mice (22 – 25 g), collected from the Biotechnology Center of Ho Chi Minh City, were used to study the laxative effect. The animals were acclimatized under laboratory conditions (relative humidity of 50–60%, temperature 25°C, and 12-h light-dark cycles) with free access to food and water for 7 days prior to the experiments. All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee of the National Institute of Medicinal Materials, Hanoi, Vietnam (Ref: 03/2024-HĐTVĐĐTĐV).

2.3. Acute oral toxicity test

Mice (n=10) were fasted overnight prior to oral administration of the extract at different concentrations via a blunt-tipped gavage needle. After administration of the extract, the animals were provided with food and water ad libitum. The mice were observed for behavioral changes, physical activity, food and water intake, excretion, and mortality for 10 days. If any animals died, a necropsy was performed to assess gross pathological changes [8-10]. The LD_{50} value was subsequently calculated using the Karber-Behrens formula:

$$\text{Log}(LD_{50}) = \text{Log}(LD_{100}) - \frac{\sum (d \times z)}{n}$$

Where: $\text{Log}(LD_{50})$: Dose causing death in 50% of experimental animals, $\text{Log}(LD_{100})$: Dose causing death in 100% of experimental animals, d: Difference between two consecutive doses ($(\text{Log}(D_{i+1}) - \text{Log}(D_i))$), z: Mean number of dead animals between two consecutive doses, n: Number of animals per group.

2.4. Evaluating the laxative effect of the extract in loperamide-induced constipated mice

The experiment was conducted for 5 days. On the day before the experiment, mice were fasted overnight with free access to water to determine their baseline body weight. To establish a reliable experimental constipation model, mice were orally administered loperamide (Nadyphar Pharmaceutical Joint Stock Company, Vietnam) at a dose of 10 mg/kg once daily for 5 days (except for the normal control group). This specific dose and duration were selected based on established protocols from previous pharmacological studies [11]. At this regimen, loperamide effectively binds to peripheral μ-opioid receptors to inhibit intestinal peristalsis and reduce fluid secretion, thereby inducing a stable and reproducible constipated state without causing severe systemic toxicity. One hour later, the mice were orally given the reference drug [12],[13] or the extract at doses of 75, 150, and 300 mg/kg. Before drug administration on day 5, mice were individually placed in metabolic cages and fasted overnight with free access to water. Fecal samples were collected during the first 12 hours (every 2 h) and during the following 12 hrs (mice were allowed normal feeding during the first 12 hours of fecal collection) [14]. The feces collected during the first 12 hrs were weighed and then dried in an oven at 50°C for 60 min. The body weight of the mice was recorded daily throughout the experiment. The mice were randomly divided into the following experimental groups (n = 8):

- Control group (Control): Distilled water.
- Constipation model group (Lope): Loperamide 10 mg/kg.
- Reference group (Bisa): Loperamide 10 mg/kg + Bisacodyl (DHG Pharmaceutical Joint Stock Company, Vietnam) 1 mg/kg.
- Test group 1 (BB75): Loperamide 10 mg/kg + Extract 75 mg/kg.
- Test group 2 (BB150): Loperamide 10 mg/kg + Extract 150 mg/kg.
- Test group 3 (BB300): Loperamide 10 mg/kg + Extract 300 mg/kg.

The evaluated parameters included daily body weight changes, food intake over 12 hrs and water consumption over 24 hrs. In addition, fecal parameters were recorded, including the number of fecal pellets, fecal morphology score (0: normal; 1: soft; 2: unformed), wet fecal weight, dry fecal weight, and fecal water content. Fecal water content (%) was calculated using the following formula:

$$\text{Fecal water content (\%)} = \frac{\text{Wet fecal weight} - \text{Dry fecal weight}}{\text{Wet fecal weight}} \times 100.$$

2.5. Evaluating the effect of the extract on gastrointestinal motility in loperamide-induced constipated mice

The experiment was conducted for 6 days. Mice were orally administered the reference drug or the extract once daily, 1 hr after receiving loperamide at a dose of 10 mg/kg. On day 5, the mice were fasted overnight with free access to water. Ten minutes after the final dose on day 6, mice in each group were orally administered activated charcoal (Nam Viet Pharmaceutical Co., Ltd., Vietnam) at a dose of 300 mg/kg with an administration volume of 10 mL/kg [15]. Twenty minutes after charcoal administration, the mice were sacrificed, and the small intestine from the pylorus to the cecum was removed. Simultaneously, the entire colon was carefully excised and opened longitudinally. To evaluate fecal retention, the number of formed fecal pellets remaining inside the colon was manually counted. Subsequently, all fecal contents within the colon were gently scraped, collected, and immediately weighed using an analytical balance to determine the total amount of retained feces (expressed in mg). The mice were divided into experimental groups (n = 8) similar to those described in the experiment above. The evaluated parameters included the distance traveled by activated charcoal in the intestine, the total amount of feces remaining in the colon, and the intestinal charcoal transit ratio, calculated as follows [15],[16]:

$$\text{Charcoal transit ratio (\%)} = \frac{\text{Distance traveled by charcoal}}{\text{Total length of the small intestine}} \times 100.$$

2.6. Evaluating the effect of the extract on gastrointestinal fluid secretion in normal mice

Before the experiment, mice were individually placed in metabolic cages and fasted overnight with free access to water. The mice were orally administered the corresponding treatments according to their respective groups. Fecal samples were collected within 4 hrs after administration of castor oil (SVA Naturals, India) or the extract and then dried at 50°C for 60 mins. The mice were subsequently sacrificed, and the intestine from the pylorus to the cecum was removed. The mice were divided into experimental groups (n = 8) as follows [16],[17]:

- Control group (Control): Distilled water.
- Reference group (CastO): Castor oil 10 mL/kg.
- Test group 1 (BB75): Extract 75 mg/kg.
- Test group 2 (BB150): Extract 150 mg/kg.
- Test group 3 (BB300): Extract 300 mg/kg.

The evaluated parameters included the number of fecal pellets; fecal morphology (0 – normal; 1 – soft; 2 – unformed); intestinal weight before squeezing; intestinal weight after squeezing; weight of intestinal contents = (intestinal weight before squeezing – intestinal weight after squeezing); and fecal water content (%), calculated as:

$$\text{Fecal water content (\%)} = \frac{\text{Wet fecal weight} - \text{Dry fecal weight}}{\text{Wet fecal weight}} \times 100.$$

2.7. Data analysis

Data were compiled and processed using Microsoft Excel 2016. Statistical analysis was performed using GraphPad Prism (8.0.2, Inc., La Jolla, CA, USA). Data were checked for normality using Anderson-Darling and Shapiro-Wilk tests. Data were compared using one-way ANOVA, followed by Tukey's post-test for variance homogeneity and Dunnett's post-test for variance heterogeneity. Results are presented as Mean ± SD (Standard Deviation) in the case of a normal distribution. Data were compared using Kruskal-Wallis followed by Dunn's post-test, and results are presented as Median ± Interquartile Range in the case of a non-normal distribution. Differences were considered statistically significant when $p \leq 0.05$. Express statistical differences as follows: ns with $p > 0.05$, * with $p < 0.05$, ** with $p < 0.01$, *** with $p < 0.001$, and **** with $p < 0.0001$.

3. Results

3.1. Acute oral toxicity of P. nil seed extract

After the preliminary test, a total of six dose levels were used in the main experiment. The minimum dose causing 100% mortality (LD_{100}) was determined to be 6.1 g/kg, while the maximum dose that caused no mortality (LD_0) was 2.0 g/kg. Based on the determined LD_0 and LD_{100} values, the LD_{50} was calculated using the Karber-Behrens formula, yielding a value of 3.96 g/kg.

Table 1. Results of the acute oral toxicity test of the *P. nil* seed extract

Group	Dose (g/kg)	n	Number of dead mice	Mortality rate (%)
I	2.0	10	0	0
II	2.5	10	1	10
III	3.2	10	1	10
IV	4.0	10	4	40
V	4.9	10	9	90
VI	6.1	10	10	100

Abnormal signs observed after administration of the *P. nil* extract included loose or watery stools occurring approximately 1.5 to 4 hours after dosing. These symptoms usually resolved within 24 hrs at all tested dose levels. No gross abnormalities were observed in internal organs, including the heart, liver, kidneys, lungs, spleen, and intestines.

3.2. Laxative effect of P. nil seed extract in loperamide-induced constipated mice

Table 2. Effects of *P. nil* seed extract on body weight, water intake, and food consumption in mice

Group	Body weight Day 1 [A] (g)	Body weight Day 6 [B] (g)	Weight change [B – A] (g)	Water intake (24h, mL)	Food intake (12h, g)
Control	22.46 ± 1.26	20.29 ± 0.75^{ns}	-2.18 ± 0.77^{ns}	6.31 ± 1.03*	2.22 ± 0.51^{ns}
Lope	22.85 ± 2.37	21.05 ± 2.43	-1.80 ± 1.05	8.78 ± 1.70	2.82 ± 0.58
Bisa	22.70 ± 2.54	20.16 ± 1.49^{ns}	-2.54 ± 1.64^{ns}	6.25 ± 1.85*	2.03 ± 0.64^{ns}
BB75	18.94 ± 6.33	16.94 ± 6.51^{ns}	-1.79 ± 0.52^{ns}	5.06 ± 0.91***	1.51 ± 0.55***
BB150	20.56 ± 1.59	18.59 ± 1.57^{ns}	-1.98 ± 0.45^{ns}	5.66 ± 1.81**	1.99 ± 0.45^{ns}
BB300	22.01 ± 1.82	19.64 ± 1.52^{ns}	-2.38 ± 1.37^{ns}	5.75 ± 1.73**	2.12 ± 0.73^{ns}

Statistical symbols indicate comparisons with the constipation model group (Lope) using Tukey's test.

The results in **Table 2** showed no statistically significant differences in the initial body weight of mice (Day 1) among the experimental groups ($p > 0.05$). After 6 days of treatment, there were also no statistically significant differences in body weight between the constipation model group and the groups treated with *P. nil* seed extract at different doses or the reference drug group ($p > 0.05$). Similarly, the changes in body weight during the experimental period did not differ significantly among the groups ($p > 0.05$). However, the 24-hour water intake in the constipation model group was significantly higher than that in the control group ($p < 0.05$). In contrast, mice treated with *P. nil* seed extract (75, 150, and 300 mg/kg) and the reference drug showed significantly lower water intake compared with the constipation model group ($p < 0.05$). Regarding food intake over 12 hrs, no statistically significant differences were observed between the constipation model group and the groups treated with the extract at doses of 150 and 300 mg/kg or the reference drug ($p > 0.05$). However, the group treated with 75 mg/kg extract showed significantly lower food intake compared with the constipation model group ($p < 0.001$).

Table 3. Effects of *P. nil* seed extract on fecal parameters

Group	Wet fecal weight (12h, g)	Dry fecal weight (12h, g)	Fecal water content (%)	Fecal pellets numbers (12h)	Fecal morphology score (12h)
Control	1.35 ± 0.87^{ns}	0.66 ± 0.43^{ns}	50.71 ± 6.43*	47.25 ± 7.05*	1 (1; 1)^{ns}
Lope	1.14 ± 0.71	0.66 ± 0.39	40.81 ± 4.89	35.25 ± 6.36	0.5 (0; 1)
Bisa	0.77 ± 0.57^{ns}	0.36 ± 0.28^{ns}	53.81 ± 5.31**	32.88 ± 8.36^{ns}	1 (0; 1)^{ns}
BB75	0.44 ± 0.30^{ns}	0.23 ± 0.16^{ns}	45.19 ± 7.57^{ns}	22.38 ± 5.42*	1 (0; 1)^{ns}
BB150	1.10 ± 0.76^{ns}	0.45 ± 0.31^{ns}	58.05 ± 8.16****	30.88 ± 9.31^{ns}	1 (0; 1)^{ns}
BB300	1.04 ± 0.85^{ns}	0.49 ± 0.41^{ns}	52.62 ± 6.41*	40.50 ± 9.67^{ns}	1 (1; 1.75)^{ns}

Statistical symbols indicate comparisons with the constipation model group (Lope) using Tukey's test, while the Dunn's test was used for fecal morphology score.

The results in **Table 3** showed that there were no statistically significant differences in wet or dry fecal weight after 12 hrs between the control group and the constipation model group, as well

as between the constipation model group and the groups treated with *P. nil* seed extract at different doses or the reference drug ($p > 0.05$). The fecal water content in the constipation model group was significantly lower than that in the control group ($p < 0.05$). In contrast, the groups treated with the extract at doses of 150 and 300 mg/kg, as well as the bisacodyl group, showed significantly higher fecal water content compared with the constipation model group ($p < 0.05$), while the 75 mg/kg extract group showed no significant difference compared with the constipation model group ($p > 0.05$). The extract at doses of 150 and 300 mg/kg increased fecal water content by approximately 12 – 18% compared with the constipation model group, showing an effect comparable to that of bisacodyl (1 mg/kg). The total number of fecal pellets within 12 hrs in the constipation model group was significantly lower than that in the control group ($p < 0.05$). No statistically significant differences were observed between the constipation model group and the groups treated with 150 and 300 mg/kg extract or the reference drug ($p > 0.05$). However, the BB75 group showed a significantly lower number of fecal pellets compared with the constipation model group ($p < 0.05$) (**Fig. 1**). The fecal morphology score after 12 hrs showed no statistically significant differences between the control group and the constipation model group, nor between the constipation model group and the treated groups ($p > 0.05$), although a trend toward higher scores was observed in the normal control and treatment groups compared with the constipation model group.

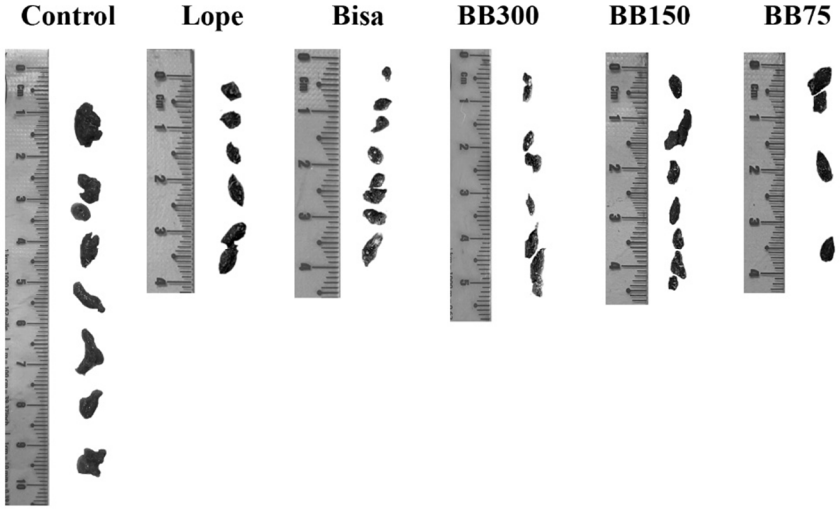

Fig. 1. Number of fecal pellets in mice after 12 hours

3.3. Effects of P. nil seed extract on gastrointestinal motility in loperamide-induced constipated mice

Table 4. Effects of *P. nil* seed extract on gastrointestinal motility

Group	Small intestine length (cm)	Charcoal transit distance (cm)	Charcoal transit ratio (%)	Number of fecal pellets remaining in colon	Amount of feces remaining in colon (mg)
Control	45.31 ± 7.26	29.88 ± 5.98****	65.86 ± 6.47*	3.75 ± 0.89*	73.66 ± 13.28*
Lope	49.63 ± 5.07	14.81 ± 4.60	29.37 ± 6.38	5.13 ± 0.83	100.83 ± 13.57
Bisa	49.81 ± 6.41	21.13 ± 5.22*	42.55 ± 10.17*	3.75 ± 0.71*	69.78 ± 21.67**
BB75	49.81 ± 4.88	14.75 ± 3.75^{ns}	29.74 ± 7.22^{ns}	3.63 ± 0.52**	53.35 ± 14.83****
BB150	45.06 ± 3.95	16.25 ± 3.37^{ns}	36.10 ± 7.06^{ns}	2.38 ± 0.92****	37.83 ± 13.11****
BB300	46.81 ± 5.58	19.63 ± 4.33*	41.90 ± 7.38*	2.13 ± 0.99****	35.09 ± 23.56****

Statistical symbols indicate comparisons with the constipation model group (Lope) using Tukey's test.

The results in **Table 4** showed that there were no statistically significant differences in the length of the small intestine among the experimental groups ($p > 0.05$). The charcoal

transit distance in the constipation model group was significantly lower than that in the control group ($p < 0.05$). In contrast, the BB300 group and the bisacodyl group showed significantly higher charcoal transit distances compared with the constipation model group ($p < 0.05$), whereas the groups treated with 75 and 150 mg/kg extract did not show significant differences compared with the constipation model group ($p > 0.05$). Similarly, the charcoal transit ratio in the constipation model group was significantly lower than that in the control group ($p < 0.05$). At the dose of 300 mg/kg, the extract enhanced intestinal motility by approximately 11% compared with the constipation model group, an effect comparable to bisacodyl (1 mg/kg); correspondingly, the BB300 and bisacodyl groups exhibited significantly higher charcoal transit ratios than the constipation model group ($p < 0.05$), whereas the BB75 and BB150 groups showed no statistically significant differences ($p > 0.05$) (**Fig. 2**). The number of fecal pellets and the fecal content remaining in the colon were significantly higher in the constipation model group than in the control group ($p < 0.05$). Treatment with *P. nil* seed extract at different doses and bisacodyl significantly reduced these parameters compared with the constipation model group ($p < 0.05$). Notably, the extract at doses of 150 and 300 mg/kg increased defecation frequency by approximately 62 – 65% relative to the constipation model group, showing a stronger effect than bisacodyl (1 mg/kg).

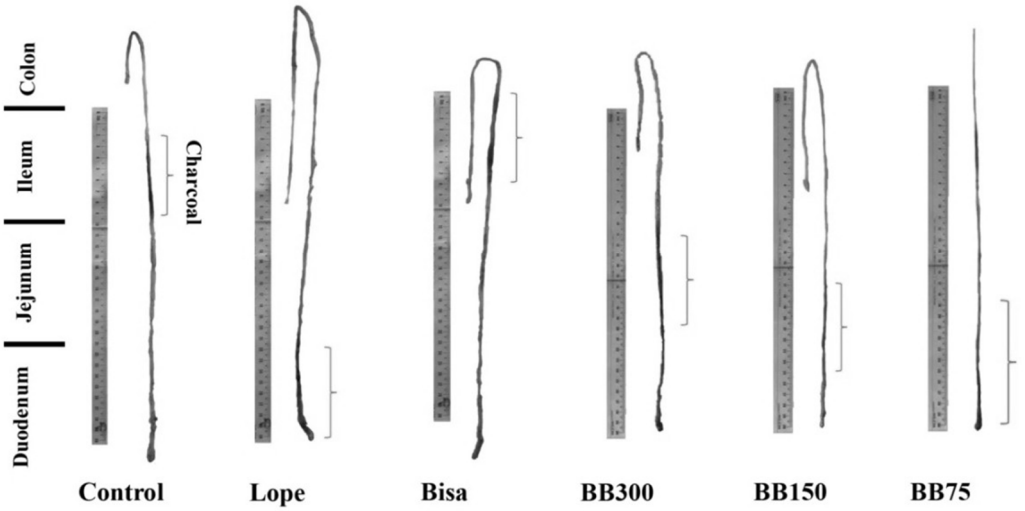

Fig. 2. Representative image showing the gastrointestinal transit of activated charcoal along the small intestine used to determine the charcoal transit distance and transit ratio in mice

3.4. Effects of P. nil seed extract on gastrointestinal secretion in normal mice

Table 5. Effects of *P. nil* seed extract on gastrointestinal secretion

Group	Number of fecal pellets (4h)	Fecal morphology score	Wet fecal weight (g)	Dry fecal weight (g)	Fecal water content (%)	Intestinal weight before squeezing (g)	Intestinal weight after squeezing (g)	Intestinal content weight (g)
Control	5.17 ± 1.17	0 (0; 1)	0.10 ± 0.03	0.07 ± 0.02	36.15 ± 3.96	1.49 ± 0.23	0.96 ± 0.28	0.54 ± 0.31
CastO	7.00 ± 2.68^{ns}	1 (0.75; 2)^{ns}	0.35 ± 0.05****	0.10 ± 0.04^{ns}	70.30 ± 8.14****	2.09 ± 0.32*	1.16 ± 0.11^{ns}	0.94 ± 0.32*
BB75	3.67 ± 2.73^{ns}	1 (0.75; 1.25)^{ns}	0.07 ± 0.05^{ns}	0.04 ± 0.03^{ns}	46.21 ± 10.89^{ns}	1.95 ± 0.34^{ns}	1.12 ± 0.25^{ns}	0.83 ± 0.15^{ns}
BB150	5.33 ± 2.34^{ns}	1 (0; 2)^{ns}	0.15 ± 0.06^{ns}	0.07 ± 0.04^{ns}	55.19 ± 8.75**	2.20 ± 0.29**	1.24 ± 0.27^{ns}	0.95 ± 0.20**
BB300	6.33 ± 1.75^{ns}	1.5 (0.75; 2)^{ns}	0.19 ± 0.07^{ns}	0.06 ± 0.03^{ns}	66.21 ± 4.28****	2.37 ± 0.38****	1.24 ± 0.28^{ns}	1.13 ± 0.11***

Statistical symbols indicate comparisons with the control group (Control) using Tukey's test, while the Dunn's test was used for fecal morphology scores.

The results in **Table 5** showed that there were no statistically significant differences in the number of fecal pellets within 4 hours between the control group and the groups treated with *P. nil* seed extract at different doses ($p > 0.05$). The number of fecal pellets in the castor oil group and the BB300 group tended to be higher than that in the control group; however, the differences were

not statistically significant ($p > 0.05$) (**Fig. 3**). The fecal morphology scores in the extract-treated groups and the reference group were higher than those in the control group, but the differences were not statistically significant ($p > 0.05$). Similarly, there were no statistically significant differences in wet or dry fecal weight between the control group and the experimental groups ($p > 0.05$). However, the fecal water content in the groups treated with 150 and 300 mg/kg extract, as well as in the castor oil group, was significantly higher than that in the control group ($p < 0.05$). The BB75 group also showed a higher fecal water content than the control group, but the difference was not statistically significant ($p > 0.05$). The intestinal weight before squeezing in the BB150, BB300, and castor oil groups was significantly higher than that in the control group ($p < 0.05$), whereas the BB75 group showed no significant difference ($p > 0.05$). The intestinal weight after squeezing did not differ significantly between the control group and the experimental groups ($p > 0.05$). In contrast, the intestinal content weight in the BB150, BB300, and castor oil groups was significantly higher than that in the control group ($p < 0.05$), representing an increase of approximately 75 – 109% compared with the control group and demonstrating an effect similar to that of the reference control (castor oil), while the BB75 group showed no statistically significant difference ($p > 0.05$).

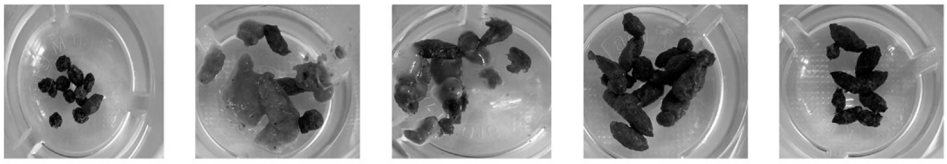

Fig. 3. Fecal morphology of mice within 4 hours

4. Discussion

The results of this study showed that the oral LD_{50} of the *P. nil* seed extract in white mice was 3.96 g/kg, indicating low acute toxicity. According to the toxicity classification proposed by Hodge and Sterner, substances with an oral LD_{50} ranging from 0.5 to 5 g/kg are categorized as having low toxicity [18]. Therefore, the *P. nil* seed extract evaluated in this study can be considered to have a relatively safe level when administered orally. Compared with the traditional medicinal dosage, *P. nil* seeds are commonly used at a dose of 4 – 8 g of crude drug per day for adults according to the Vietnamese Pharmacopoeia V [2]. When converted to the equivalent dose in mice using the body surface area conversion factor, this corresponds to approximately 127 – 254 mg/kg [8]. Thus, the LD_{50} value (3.96 g/kg) is about 15.6 times higher than the maximum experimental dose, indicating a relatively wide safety margin between the therapeutic dose and the acute toxic dose. This finding is consistent with the long-standing use of this medicinal plant in traditional medicine as a purgative and laxative agent.

These findings are also consistent with several previous studies on *Pharbitidis Semen*. Pharmacological studies have shown that the major active constituents of this medicinal material are resin glycosides (pharbitin), which possess strong biological activity but exhibit relatively low acute toxicity when administered orally at therapeutic doses. According to the study by Ono (2017), resin glycosides isolated from plants of the Convolvulaceae family can stimulate intestinal motility and increase intestinal fluid secretion, while generally causing minimal systemic toxicity when used at appropriate doses [4]. An interesting observation in this study was that, at higher doses, mice exhibited loose or watery stools within 1.5 – 4 hrs after administration, with recovery occurring after approximately 24 hours. This phenomenon is consistent with the pharmacological characteristics of resin glycosides, which are known to stimulate the intestinal mucosa and increase intestinal fluid secretion, thereby producing purgative or laxative effects [3]. Therefore, these manifestations are more likely related to the pharmacological activity of the extract rather than true toxic effects. In addition, no gross pathological alterations were observed in major internal organs, including the liver, kidneys, heart, lungs, and spleen, suggesting that the *P. nil* seed extract did not cause significant acute toxicity to target organs under the

experimental conditions. However, it should be noted that acute toxicity studies only reflect short-term effects. Therefore, further investigations on subacute and chronic toxicity are necessary to provide a more comprehensive evaluation of the safety profile of this medicinal plant.

In the present study, a loperamide-induced constipation model was used to evaluate the laxative effect of *P. nil* seed extract. Loperamide is a peripheral μ-opioid receptor agonist that can inhibit intestinal motility, prolong fecal retention time in the colon, and reduce intestinal fluid secretion, thereby inducing experimental constipation. Consequently, this model is widely used in studies evaluating laxative agents [13],[14],[15]. A noteworthy physiological response observed in our loperamide-induced constipation model was the apparent paradox between water intake and fecal water content. Although the constipation model group consumed significantly more water than the control group (Table 2), their fecal water content remained significantly lower (Table 3). This phenomenon can be fundamentally explained by the pharmacological action of loperamide. By agonizing peripheral μ-opioid receptors, loperamide profoundly inhibits intestinal fluid secretion and peristalsis. This prolongs colonic transit time, maximizing water reabsorption and resulting in dry, hard feces. The significant increase in water intake is likely a systemic compensatory mechanism; the animals drink more to compensate for the localized lack of luminal fluid and the difficulty in passing hard stools. However, because loperamide effectively blocks the secretory pathways into the gut lumen, the excess ingested water is absorbed systemically and excreted via the kidneys rather than hydrating the fecal mass. This observation strongly validates the successful establishment and robustness of the experimental constipation model used in this study. The results showed that the *P. nil* seed extract at doses of 150 and 300 mg/kg significantly increased fecal water content compared with the constipation model group. In addition, at the dose of 300 mg/kg, the extract also significantly increased the intestinal charcoal transit ratio and reduced fecal retention in the colon. These findings indicate that the extract can improve gastrointestinal motility and promote fecal excretion, thereby exhibiting a clear laxative effect. These results are consistent with several previous studies on medicinal plants with laxative activity. A study by Wintola et al. (2010) using a loperamide-induced constipation mouse model demonstrated that *Aloe ferox* extract significantly increased fecal water content and improved intestinal motility, showing a mechanism similar to that observed in the present study [13]. In addition, Lim et al. (2019) reported that fermented barley extract could enhance gastrointestinal transit and improve loperamide-induced constipation [14]. An interesting and noteworthy observation from our data is that the low-dose group (BB75) exhibited a significantly lower number of fecal pellets compared to the loperamide-induced constipation model group (Lope). This seemingly paradoxical result could be attributed to several intersecting pharmacological and physiological factors. Firstly, as shown in Table 2, mice in the BB75 group consumed significantly less food compared to the Lope group ($p < 0.001$). A reduction in food intake directly limits fecal mass formation, resulting in fewer fecal pellets. Secondly, at the dose of 75 mg/kg, the concentration of the primary laxative constituents, such as resin glycosides, might be sub-therapeutic and insufficient to overcome the potent μ-opioid receptor agonism of loperamide. Furthermore, *P. nil* seeds are known to contain various polyphenols. It is hypothesized that at low doses, the mildly astringent properties of certain polyphenols might manifest before the strong purgative effect of resin glycosides reaches its therapeutic threshold at higher doses (150 and 300 mg/kg). Furthermore, visual observation of the fecal morphology (Fig. 1) in the BB75 group reveals bulkier, aggregated fecal masses rather than distinct pellets. This physical impaction is consistent with prolonged colonic retention and delayed transit time, allowing the limited fecal matter to coalesce into larger, drier clumps due to the unresolved loperamide-induced stasis. This suggests a potential biphasic dose-response relationship and strongly highlights the necessity of dosage optimization when utilizing this herbal extract in clinical practice.

The laxative mechanism of *P. nil* seeds is believed to be mainly associated with resin glycosides (pharbitin). These compounds can stimulate the intestinal mucosa, increase secretion of water and electrolytes into the intestinal lumen, and enhance gastrointestinal smooth muscle contractions, thereby softening stools and promoting intestinal motility. An *in vitro* study by Zhou et al. (2022) demonstrated that pharbitin could affect the contractile tone of

the colon and ileum, providing further evidence for the laxative activity of this medicinal plant [3]. However, in the present study, the tested extract was not directly characterized for pharbitin by TLC, HPLC, or other dedicated analytical methods. These should be considered for future research to obtain more convincing conclusions about the link between pharbitin and laxative effect. In this study, the 70% ethanol extraction solvent was selected for its intermediate polarity, which is optimal for recovering a broad spectrum of bioactive compounds, including the primary purgative resin glycosides (pharbitin) and various polyphenols. Furthermore, this solvent is safe, easily recoverable, and suitable for subsequent pharmacological and formulation studies.

To ensure the reproducibility and quality of the extract, chlorogenic acid (CGA) and isochlorogenic acid A (ICGA) were employed as chemical markers, as resin glycosides possess complex structures that present challenges for routine quantification. Quantitative analysis using the HPLC-DAD method developed in our previous study confirmed that the extract batch contained 1.20 ± 0.04% of chlorogenic acid and 0.65 ± 0.01% of isochlorogenic acid A. These compounds not only serve as reliable markers for standardization but also support the extract's biological profile through antioxidant mechanisms and protection of the intestinal mucosa. Moreover, the results obtained in normal mice demonstrated that the extract at doses of 150 and 300 mg/kg significantly increased both fecal water content and intestinal content weight, indicating a strong capacity to stimulate fluid secretion into the intestinal lumen. To validate this secretory assay, castor oil was employed as a positive control. Upon oral administration, castor oil is rapidly hydrolyzed by intestinal lipases into its active metabolite, ricinoleic acid. This compound acts as a potent mucosal irritant that alters epithelial permeability and triggers a massive net secretion of water and electrolytes into the gut lumen while inhibiting their reabsorption [19]. Consistent with this well-established mechanism, the castor oil group in our study exhibited profound increases in luminal fluid accumulation. The comparable secretory responses observed in the *P. nil* extract-treated groups strongly suggest that the extract exerts its laxative efficacy through a similar mechanism of enhancing intestinal fluid secretion. This mechanism is similar to that of stimulant laxatives, such as bisacodyl, which act by enhancing intestinal secretion and stimulating intestinal motility [20].

Beyond the direct mucosal stimulation by resin glycosides, the laxative efficacy of *P. nil* seed extract may involve broader metabolic and neurogastroenterological pathways. Recent evidence highlights that loperamide-induced constipation is strongly associated with bile acid dysregulation, characterized by the excessive accumulation of bile acids in the liver and a consequent reduction in their fecal excretion [21]. Since luminal bile acids are potent physiological stimulants for colonic secretion and motility, enhancing their excretion is a key mechanism for resolving constipation. Furthermore, another crucial mechanism underlying intestinal motility involves the 5-hydroxytryptamine (5-HT) signaling pathway. Constipation typically disrupts this pathway, significantly increasing 5-HT levels in the serum while drastically decreasing them in the intestinal tissue, thereby impeding the peristaltic reflex [22]. The significant improvements in gastrointestinal transit and fluid secretion observed in our extract-treated mice (BB150 and BB300) strongly suggest that *P. nil* seeds might exert regulatory effects on the enteric nervous system, potentially by restoring intestinal 5-HT synthesis and promoting bile acid excretion into the colon.

At the tissue level, the histological integrity of the colon mucosa is a primary determinant of normal bowel function. In loperamide-induced models, prolonged fecal impaction structurally alters the colonic mucosa and reduces mucin production, leading to the evident presence of hard, dry fecal pellet remnants compacted within the colon lumen [14]. In our study, the profound reduction in both the number and mass of retained feces in the colon following *P. nil* extract administration (Table 4) indicates its high efficacy in clearing these luminal remnants. While the current findings clearly demonstrate physiological improvements in transit and fluid secretion, subsequent histological and morphometric observations of the colon mucosa, such as quantifying goblet cells and mucin layer thickness, will provide deeper structural insights into how the extract protects the intestinal barrier against impaction-induced damage.

Overall, the findings of this study are consistent with previously published pharmacological data on *P. nil* seeds, further supporting the scientific basis for the traditional use of this medicinal plant in the management of

constipation. However, to better clarify the mechanism of action, future studies should focus on the isolation of active compounds and the evaluation of their effects on the enteric nervous system as well as intestinal water and electrolyte transport pathways.

5. Conclusions

The 70% ethanol extract of *P. nil* seeds has acute oral toxicity in *Swiss albino* mice, with an LD_{50} value of 3.96 g/kg. Pharmacologically, the extract exerts significant laxative and ameliorative effects against loperamide-induced constipation. These therapeutic benefits are primarily mediated by stimulating intestinal fluid secretion and enhancing gastrointestinal motility, which effectively relieve fecal impaction. Overall, these findings provide robust scientific validation for the traditional use of *P. nil* seeds in the prevention and management of constipation, highlighting its promising potential for development into standardized herbal therapeutics.

Acknowledgement: *This work was partially funded by Traphaco Joint Stock Company under the project "Study on chemical composition and some biological effects, development of a standardized extraction process and standardization of Morning Glory seeds (Semen Pharbitidis)", Project code: 09-2023/HĐNCKH-BB, dated May 26, 2023.*

References

1. Do H. B. (2016), *Medicinal plants and animals used as medicines in Vietnam, Vol. 1 (in Vietnamese)*, Science and Technology Publishing House, Hanoi, 207–208.
2. Ministry of Health (Vietnam) (2017), *Vietnamese Pharmacopoeia V (in Vietnamese)*, Medical Publishing House, Hanoi.
3. Zhou J. R., Tokutomi N., Satou Y., Yasuda S., Kinoshita H., Nohara T., Yokomizo K., Ono M. (2022), Different effects on the tonus of colon and ileum isolated from mouse by resin glycoside (pharbitin) of *Pharbitidis Semen*, *BMC Complementary Medicine and Therapies*, 22(1), 82.
4. Ono M. (2017), Resin glycosides from Convolvulaceae plants, *Journal of Natural Medicines*, 71(4), 591–604.
5. Wang Q., Sun Y., Yang B., Wang Z., Liu Y., Cao Q., Sun X., Kuang H. (2014), Optimization of polysaccharides extraction from seeds of *Pharbitis nil* and its antioxidant activity, *Carbohydrate Polymers*, 102, 460–466.
6. Kim K. H., Woo K. W., Moon E., Choi S. U., Kim S. Y., Choi S. Z., Son M. W., Lee K. R. (2014), Identification of antitumor lignans from the seeds of morning glory (*Pharbitis nil*), *Journal of Agricultural and Food Chemistry*, 62(31), 7746–7752.
7. Nguyen T. K. O., Ly H. T., Le V. M., Nguyen M. K., Nguyen T. H., Vu H. T., Nguyen H. V., Tran Q. L., Nguyen T. V. H., Do T. H. (2025), *Pharbitis nil* (L.) Choisy seed extract attenuates cyclophosphamide-induced immunosuppression via regulation of Th2-type immune and humoral immune responses, *Advances in Traditional Medicine*, doi: 10.1007/s13596-025-00859-1.
8. Ministry of Health (Vietnam) (2015), *Decision No. 141/QĐ-K2ĐT dated October 27, 2015: Guidelines for Preclinical and Clinical Trials of Traditional and Herbal Medicines (in Vietnamese)*, Department of Science and Training, Ministry of Health, Vietnam.
9. Do T. D. (2014), *Methods for determining drug toxicity (in Vietnamese)*, Medical Publishing House, Hanoi.
10. Institute of Medicinal Materials (Vietnam) (2016), *Methods for studying the pharmacological effects of drugs derived from herbal medicines (in Vietnamese)*, Science and Technology Publishing House, Hanoi, 140–149.
11. Wang L., Xie S., Jiang X., Xu C., Wang Y., Feng J., Yang B. (2023), Therapeutic effects of *Bombax ceiba* flower aqueous extracts against loperamide-induced constipation in mice, *Pharmaceutical Biology*, 61(1), 125–134.
12. Ayele A. G., Mulugeta B., Wondmkun Y. T. (2023), Evaluation of the *in vivo* laxative effects of aqueous root extracts of *Euclea racemosa* L. In mice, *Metabolism Open*, 17, 100222.
13. Wintola O. A., Sunmonu T. O., Afolayan A. J. (2010), The effect of *Aloe ferox* Mill. In the treatment of loperamide-induced constipation in Wistar rats, *BMC Gastroenterology*, 10, 95.
14. Lim J. M., Kim Y. D., Song C. H., Park S. J., Park D. C., Cho H. R., Jung G. W., Bashir K. M. I., Ku S. K., Choi J. S. (2019), Laxative effects of triple fermented barley extracts (Fbe) on loperamide (LP)-induced constipation in rats, *BMC Complementary and Alternative Medicine*, 19(1), 143.
15. Choi J. S., Kim J. W., Cho H. R., Kim K. Y., Lee J. K., Sohn J. H., Ku S. K. (2014), Laxative effects of fermented rice extract in rats with loperamide-induced constipation, *Experimental and Therapeutic Medicine*, 8(6), 1847–1854.
16. Ayele A. G., Kawet J. S. (2024), Evaluation of the *in vivo* laxative effects of aqueous leaf and stem extracts of *Artemisia abyssinica* in mice, *Journal of Experimental Pharmacology*, 16, 135–142.
17. Saito T., Mizutani F., Iwanaga Y., Morikawa K., Kato H. (2002), Laxative and anti-diarrheal activity of polycarbophil in mice and rats, *Japanese Journal of Pharmacology*, 89(2), 133–141.
18. Hodge H. C., Sterner J. H. (1949), Tabulation of toxicity classes, *American Industrial Hygiene Association Quarterly*, 10(4), 93–96.
19. Tunaru S., Althoff T. F., Nüsing R. M., Diener M., Offermanns S. (2012), Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors, *Proceedings of the National Academy of Sciences of the United States of America*, 109(23), 9179–9184.
20. Corsetti M., Landes S., Lange R. (2021), Bisacodyl: A review of pharmacology and clinical evidence to guide use in clinical practice in patients with constipation, *Neurogastroenterology and Motility*, 33(10), e14123.
21. Lei Z., Rong H., Yang Y., Yu S., Zhang T., Chen L., Nie Y., Song Q., Hu Q., Guo J. (2022), Loperamide induces excessive accumulation of bile acids in the liver of mice with different diets, T*oxicology*, 477, 153278.
22. 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-hydroxytryptamine signaling by *Ligilactobacillus salivarius* Li01, *Nutrients*, 14(19), 4083.