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Evaluation of In Vivo Antidiarrheal Activities of Hydroalcoholic Leaf Extract of *Dodonaea viscosa* L.(Sapindaceae) in Swiss Albino Mice

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Abstract

Traditionally people used *Dodonaea viscosa* for the treatment of various ailments, including diarrhea. Therefore, this study was aimed to evaluate the antidiarrheal activity of the 80% methanolic leaf extract of *D viscosa* against castor oil-induced diarrhea in mice models. Different doses of 80% methanolic leaf extract of *D viscosa* (100, 200, and 400 mg/kg) were evaluated for their antidiarrheal activities using castor oil–induced diarrhea, gastrointestinal transit, and enteropooling models in Swiss albino mice. At all test doses, the plant extract showed significant ($P < .05$) inhibition in the frequency of defecation of wet feces and total fecal output as compared to the control group. Similarly, at all dose ranges used the plant extract demonstrated significant ($P < .05$) reduction in an intraluminal fluid accumulation as compared to the untreated group. Besides, at higher doses, the plant extract also indicated significant ($P < .05$) antimotility activity in comparison with the control. In conclusion, these findings illustrated that the 80% methanolic leaf extract of *D viscosa* supported the traditional claim of antidiarrheal activity of the plant though further investigations are warranted.

Keywords

Dodonaea viscosa, antidiarrhea, castor oil, enteropooling, gastrointestinal motility

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Diarrheal illnesses are one of the main reasons for morbidity and mortality in developing nations and are accountable for the death of hundreds of thousands of people every year.[1] A report in 2015 demonstrated that diarrhea is one of the main killers of children that accounts for 9% of all deaths among kids below the age of 5 years worldwide.[2] According to this report, sub-Saharan Africa and southern Asia were recorded as the regions that experienced the highest child death toll as a result of diarrhea.[3] In Ethiopia, diarrheal disease is a major public health problem, and it is also one of the top 15 countries in which nearly three-fourths of child deaths occur due to diarrhea.[4] Overall, the prevalence of the diarrheal disease still stays high no matter how much the attempts were made via many governments and worldwide groups to reduce it.[5]

Irrespective of great technological development in modern medicine, 80% of human beings in the growing nations rely on healing practices and medicinal plants for their daily health care needs.[6] Similarly, plants have traditionally been used as a supply of drugs in Ethiopia since a long time to manipulate several illnesses afflicting human beings and their livestock.[7] The use of herbal medicine is getting popularized in developing and advanced nations because of its natural origin and lesser adverse effects.[8] Natural products have additionally been a success in drug development and over 50% of the best-selling prescription drugs in use at this time derived from herbal products.[9] Therefore, the World Health Organization (WHO) encouraged researches for the treatment and prevention of diarrheal illnesses depending on traditional medical practice.[10] The use of traditional medicines to combat the consequences of diarrhea has been employed by WHO in its Diarrhea Control Program.[11] Adverse effects related to opioid-like antimotility drugs are restricting their uses and pushing researchers to search for new antidiarrheal compounds with diverse chemical systems and novel mechanisms of action. Therefore,

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researchers are increasingly turning their attention to folk medicine, searching out new leads to broadening options of drugs toward diarrheal diseases.[12,13]

Dodonaea viscosa (*Sapindaceae*) has different names in Ethiopia according to local languages spoken in the country: kitkita (in Ahmaric), itacha (in Afan Oromo), kerara (in Agewugna), itancha (in Sidamagna), den or hayramat (in Somaligna), and geregetwa (in Wolaytegna).[14] The plant is traditionally used in folk medicine to treat various ailments afflicting human beings. Accordingly, in African countries and other Asian countries, people administer the dried leaves decoction for the treatment of stomach ulcer after grinding and mixing with milk or honey, hemorrhoids, stomachache, and pains of hepatic or splenic origin.[15] In South Africa, people use the plant leaves decoction for the treatment of common colds, influenza, bladder and kidney problem, pain, headache, and fatigue.[16] In Oman, the leaves are used to treat itching and rash, swelling, rheumatoid arthritis, bone disorders, gastrointestinal disorder and muscle relaxation problem; as well as roots are also used to treat ulcer and headache.[17] However, the mixtures of leaves and roots as paste are used to treat dental pain, headache, indigestion, diarrhea, and constipation.[18] In Pakistan, the leaves of *D viscosa* are used to treat joint pain.[19] Moreover, people in Australia have been used the plant for the treatment of a wound, bleeding, bone fractures, and snake bites whereas in India, the plant is used to treat different ailments like bone fractures, snake bites, wound healing, headache, indigestion, and diarrhea.[20] The plant leaf has also been used traditionally in Ethiopia for the treatment of evil eye, diarrhea, and ticks[21] as well as for anthrax, stabbing pain, and sun problem.[22]

The *D viscosa* leaves have been evaluated for its various activities and provided promising effects. Accordingly, the plant leaves demonstrated antidiabetic effects against alloxan-induced diabetes in rabbits and rats,[23,24] antibacterial activities against selected gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, and *Staphylococcus aureus*), and gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*),[25] antifungal activities against different species of fungi,[26,27] significant anti-inflammatory effect against the paw edema induced by carrageenan injection,[28] and antinociceptive activity in rats and mice against experimental pain models of glacial acetic acid–induced writhing, hot plate, and tail flick.[29] The plant extract has also exhibited antiulcer activity against ethanol and indomethacin-induced gastric ulcer in Wistar rats,[30] as well as an antidiarrheal effect against castor oil-induced diarrhea in mice in which alcohol and aqueous extracts of the roots of the plant were significantly reduced diarrhea in mice.[20] Moreover, the plant has also been evaluated in Ethiopia for its antimalarial activities,[31] antimicrobial and anti-inflammatory effects.[32] Despite its traditional claims, the efficacy of the leaves of *D viscosa* against diarrhea is not yet scientifically validated. Therefore, this study was aimed to evaluate the antidiarrheal activity of hydroalcoholic (80% methanolic) leaf extract of *D viscosa* against castor oil–induced diarrhea in mice.

Methods

Drugs and Chemicals

The solvent used for the extraction process is of laboratory grade. Drugs and chemicals used in the study include the following: castor oil (Amman Pharmaceutical Industries, Jordan), activated charcoal (Laboratory reagent, Labchem Industries, India), loperamide HCl (Bafna Pharmaceuticals, Ltd, India), dimethyl sulfoxide (DMSO) (Blulux Laboratories, India), absolute methanol (Sigma-Aldrich, Chemie GmbH, Germany), chloroform (Sigma-Aldrich, Chemie GmbH, Germany), glacial acetic acid (BDH Ltd, England), sulfuric acid (Farm Italia Carlo Erba, Italy), ammonia (Merck Millipore, India), hydrochloric acid (BDH Ltd, England), acetic anhydride (May and Baker Ltd, Dagenham, England), ferric chloride (BDH Ltd, England), Mayer's and Dragendorff's reagents (May and Baker Ltd, Dagenham, England).

Collection of Plant Materials and Preparation

The leaves of *D viscosa* were collected in October 2018 from Beha Biftu Kebele, Shenan Dhugo Woreda (earlier Mesela Woreda), West Haraghe Zone, Oromia region, which is 404.5 km to the East of Addis Ababa, the capital of Ethiopia. The plant was authenticated by a taxonomist and a voucher specimen was deposited at the Herbarium of the College of Natural and Computational Sciences, Haramaya University for future reference. The leaves were washed gently and dried at room temperature under shade for 2 weeks. The dried leaves were then reduced to the appropriate size using mortar and pestle.

Extraction of the Plant Material

A method stated by Handa et al[33] was used for the plant extraction. Accordingly, a total of 200 g of coarsely powdered leaves were macerated with 80% methanol (80ME) in Erlenmeyer flask for 72 hours at room temperature. The extraction process was facilitated by using a mini-orbital shaker at 120 rpm with occasional stirring. After 72 hours, the extract was separated from the marc by filtering with double layered muslin cloth followed by Whatman No.1 filter paper. The marc was then remacerated twice using fresh solvents to exhaustively extract the plant material. After exhaustive extraction, the 80ME was removed by evaporation under reduced pressure using a rotary evaporator (Buchi Rotavapor, Switzerland) in distillation flask at 72 rpm and 40°C to concentrate the extract whereas the residual aqueous solvent was removed and concentrated in an oven set at 40°C. The percentage yield of 80ME was found to be 18.5% (w/w). The dried extract was weighed and transferred into an airtight container and kept in the refrigerator until the completion of the experiment.

Preliminary Phytochemical Analysis

The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, steroidal compounds, glycosides, phenols, and saponins using the procedure described by Sofowara.[34]

Experimental Animals

Swiss albino mice of either sex weighing 20 to 30 g and aged 6 to 8 weeks were used for the experiment. The mice were obtained from the animal house of the College of Natural and Computational Sciences, Haramaya University. The animals were kept in plastic cages at 22 ± 3°C and on a 12-hour light/dark cycle with access to pellet food and water *ad libitum*. Good hygiene was maintained by constant cleaning and removal of feces from cages thrice a week. The mice were acclimatized to laboratory conditions for 1 week prior to the experiment. Food was withdrawn 18 hours prior to the beginning of all the experiments. However, water was accessed except in enteropooling, where both food and water were withdrawn. The care and handling were according to international guidelines for the use and maintenance of experimental animals.[35,36]

Grouping and Dosing of Animals

Mice were randomly assigned into 5 groups of 3 extracts treated and 2 control groups with 5 mice per group. All groups received their respective treatments using oral gavage. The first group was assigned as negative control and received 10ml/kg of DMSO, the second, third, and fourth groups received 100, 200, and 400 mg/kg of extract, respectively, whereas the fifth group is assigned as positive control and received 3 mg/kg of loperamide. The doses of 80ME were determined as to 100, 200, and 400 mg/kg based on acute toxicity test.[37] That is, 1/10th of 2000 mg/kg of the dose used in acute toxicity test is used to determine the middle dose, and one-half of and 2 times the middle dose was used to determine the lowest and highest doses, respectively. The extract was reconstituted with DMSO at appropriate concentrations and a freshly prepared solution was administered for animals on the day of the experiments. Similarly, loperamide used for the positive control was also reconstituted in DMSO and administered to the animals. The volume given for both extract- and loperamide-treated groups is obtained by calculating mg/kg required for each animal and then calculating the equivalent volume that contain the desired mg of extract or loperamide from reconstituted solution for each animal.

Acute Oral Toxicity Test

According to the Organization for Economic Cooperation and Development (OECD) guidelines 2008: 425, a single female mouse was fasted for 3 hours and was loaded with 2000 mg/kg of the 80ME as a single dose by oral gavage. Based on the results of the first mouse; another 4 female mice were recruited and fasted for 3 hours and given with the same doses. The animals were observed individually for any sign of toxicity such as behavioral changes in feeding, water intake, locomotor activity, lethargy, grooming, other signs of weakness or distress or death once during the first 30 minutes, then periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter for a total of 14 days.[37]

Determination of Antidiarrheal Activity

Castor Oil–Induced Diarrhea. This study was induced by the mouse model of diarrhea using castor oil as explained by Shoba et al[38] and slightly modified by Sisay et al.[39] Swiss albino mice of either sex fasted for 18 hours, and randomly distributed into 5 groups of 5 animals per group. All animals received 0.5 mL of castor oil after 1 hour of administration of the respective doses of extract and loperamide for each animal as described under grouping and dosing section. Then each mouse was individually placed in a plastic cage where the floor was lined with a white paper. Thereafter, the animals were continuously observed for a period of 4 hours, during which the onset of diarrhea, frequency of defecation, and the weight of fecal output (wet and total feces in gram) were recorded for each mouse. The percentages of diarrheal inhibition and weight of fecal output were determined according to the formulae 1 to 3[40,41]:

$$\%\ \text{inhibition} = \left(\frac{\text{Average number of WFC} - \text{Average number of WFT}}{\text{Average number of WFC}}\right) \times 100 \quad (1)$$

where, WFC = wet feces in the control and WFT = wet feces in the test group.

$$\text{Percentage of wet fecal output} = \left(\frac{\text{Mean weight of wet feces of each group}}{\text{Mean weight of wet feces of control}}\right) \times 100 \quad (2)$$

$$\text{Percentage of total fecal output} = \left(\frac{\text{Mean weight of total feces of each group}}{\text{Mean weight of total feces of control}}\right) \times 100 \quad (3)$$

Castor Oil–Induced Gastrointestinal Motility. Twenty-five mice fasted for 18 hours and randomly allocated into 5 groups in which each group contain 5 animals and treated as described in animal grouping and dosing section. After 1 hour of extracts and loperamide administration, each mouse received 0.5 mL of castor oil orally. Again after 1 hour of administration of castor oil, each mouse received 1 mL of marker (5% activated charcoal suspension in distilled water) orally. After 1 hour of administration of activated charcoal, all animals were sacrificed, and each mouse small intestine was dissected out from pylorus to cecum and placed on a clean surface. The small intestine was carefully inspected and the distance traveled by the charcoal meal from the pylorus was measured and expressed as a percentage of the total length of the small intestine from the pylorus to the cecum (peristaltic index) as shown in formula 4. The percentage of inhibition was then expressed using formula 5[42]:

$$\text{Peristaltic index} = \left(\frac{\text{Distance traveled by charcoal meal}}{\text{The whole length of small intestine}}\right) \times 100 \quad (4)$$

$$\%\ \text{inhibition} = \left(\frac{\text{PIC} - \text{PIT}}{\text{PIC}}\right) \times 100 \quad (5)$$

where PIC = peristaltic index of control and PIT = peristaltic index of test group.

Castor Oil–Induced Enteropooling Activity. Intraluminal fluid accumulation was determined using the method described by Adeyemi and Akindele.[43] Twenty-five mice of either sex were deprived of both food and water for 18 hours and randomly allocated into 5 groups in which each group has 5 animals. Each animal received 0.5 mL of castor oil orally 1 hour after the administration of extract and loperamide as described in the grouping and dosing section. All mice were sacrificed by cervical dislocation after 1 hour of castor oil administration. Then the abdomen of each mouse was opened and the small intestine was removed, after ligation at the pyloric end and ileocecal junction, and weighed. The intestinal contents were squeezed into a graduated tube

and the volume was determined. The small intestine was reweighed and the difference between full and empty intestine was calculated. Finally, the percentage inhibitions of the volume and weight of intestinal contents were determined according to formulae 6 and 7, respectively[44]:

$$\text{Percentage of inhibition} = \left(\frac{\text{MVICC} - \text{MVICT}}{\text{MVICC}}\right) \times 100 \quad (6)$$

where, MVICC = mean volume of the intestinal content of the control group and MVICT = mean volume of the intestinal content of the test group.

$$\text{Percentage of inhibition} = \left(\frac{\text{MWICC} - \text{MWICT}}{\text{MWICC}}\right) \times 100 \quad (7)$$

where MWICC = mean weight of the intestinal content of the control group and MWICT = mean weight of the intestinal content of the test group.

The In Vivo Antidiarrheal Index. The *in vivo* antidiarrheal index (ADI) for the 80ME and standard drug was determined by using the formula 8 described by Hussain et al[45] and Than et al[46]:

$$\text{ADI} = \sqrt[3]{(Dfreq \times Gmeq \times Pfreq)} \quad (8)$$

where Dfreq = delay in defecation time or diarrheal onset (in % of negative control) and is calculated using formula 9:

$$\text{Dfreq} = \left(\frac{\text{Mean onset of diarrhea in treated group} - \text{Mean onset of diarrhea in negative control}}{\text{Mean onset of diarrhea in the negative control group}}\right) \times 100 \quad (9)$$

*G*meq is the charcoal meal travel reduction (as % of negative control) from the gastrointestinal motility test model, and *P* freq is the reduction in the number of wet stools (as % of the negative control) from the castor oil–induced diarrhea model.

Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM) for each group. The data were analyzed using SPSS software version 16.0. All the grouped data were statistically evaluated and the significance of various treatments was calculated using a one-way analysis of variances (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. A P value <.05 was considered statistically significant.

Results

Preliminary Phytochemical Screening of the Leaves of D viscosa

Preliminary phytochemical screening of the 80ME extract of the leaf of *D viscosa* showed the presence of alkaloid, flavonoids, phenolic, triterpenoids, and tannins (Table 1).

Table 1. Phytochemical Constituents of 80% Methanol Extract (80ME) of the Leaves of *Dodonaea viscosa*.

Phytochemical Tested	80ME Leaf Extract[a]
Alkaloids	+
Flavonoids	+
Phenolics	+
Saponins	+
Tannins	+
Triterpenoids	+
Steroids	−
Glycosides	+

[a]"+" indicates present and "−" insdicates absent.

Effects of D viscosa Leaf Extract on Castor Oil–Induced Diarrheal Model

The 80ME extract of the leaf of *D viscosa* significantly delayed ($P < .05$) the onset of diarrhea at all test doses of the extract administered for the animals. Similarly, the frequency of defecation was also significantly ($P < .001$) reduced by all dose ranges of the plant extract as compared to the control group. In addition, at all test doses, the plant extract was significantly ($P < .001$) decreased the average weight of wet feces and the average weight of total fecal output relative to the control group. The highest percentage of inhibition of defecation was observed at a dose of 400 mg/kg (78.57%) of 80ME leaf extract of the plant, which is comparable with percentage inhibition of standard drug loperamide (82.14%) (Table 2).

Effects of D viscosa Leaf Extract on Castor Oil–Induced Gastrointestinal Transit

In the negative control group, the distance traveled by the charcoal meal was 38.98 ± 3.06 and its peristaltic index was 77.77% ± 5.29%. The plant extract was able to significantly reduced the distance traveled by the charcoal meal at doses of 200 mg/kg (12.00 ± 4.97, $P < .01$) and 400 mg/kg (9.5 ± 4.03, $P < .01$). However, at a dose of 100 mg/kg, the plant extract did not show statistically significant inhibition of the propulsion of the charcoal marker as compared with the negative control group. The standard drug, loperamide, showed a significant reduction (8.6 ± 5.28, $P < .01$) in the distance traveled by the charcoal meal and resulted in the highest percentage of inhibition (81.5%) as compared with the negative control group (Table 3).

Effects of D viscosa Leaf Extract on Gastrointestinal Fluid Accumulation

The volume of intestinal contents and weight of intestinal contents of the negative control were 0.86 ± 0.12 and 1.19 ±

Table 2. Antidiarrheal Effect of 80% Methanol Extract (80ME) of the Leaves of *Dodonaea viscosa* on Castor Oil–Induced Diarrhea.[a]

Dose (mg/kg)	Onset of Diarrhea (min)	Number of Wet Feces	Total Number of Feces	Average Weight of Wet Feces (g)	Average Weight of Total Feces (g)	% of Inhibition of Defecation	%WWFO	%WTFO
Control	83.4 ± 9.48	5.6 ± 0.87	6.8 ± 0.86	1.23 ± 0.16	1.39 ± 0.18	—	—	—
80ME 100	152.4 ± 19.77[b]*	1.8 ± 0.20[b]**	3.2 ± 0.80[b]*	0.18 ± 0.05[b]**	0.22 ± 0.06[b]**	67.86	14.63	15.83
80ME 200	189.8 ± 11.36[b]**	1.4 ± 0.24[b]**	2.2 ± 0.37[b]**	0.13 ± 0.04[b]**	0.21 ± 0.05[b]**	75.00	10.57	15.11
80ME 400	200.6 ± 4.63[b]**	1.2 ± 0.2[b]**	1.8 ± 0.37[b]**	0.11 ± 0.01[b]**	0.20 ± 0.04[b]**	78.57	8.94	14.39
Loperamide 3	191.4 ± 12.82[b]**	1.0 ± 0.00[b]**	1.6 ± 0.24[b]**	0.09 ± 0.05[b]**	0.13 ± 0.05[b]**	82.14	7.32	9.35

Abbreviations: WWFO, weight of wet fecal output; WTFO, weight of total fecal output.
[a]Values are given as mean ± standard error of the mean (n = 5); analysis was done using one-way analysis of variance followed by Tukey honestly significant difference (HSD) post hoc test.
[b]Compared with negative control values.
*$P < .01$, **$P < .001$.

Table 3. Effect of 80% Methanol Extract (80ME) of the Leaves of *Dodonaea viscosa* on Gastrointestinal Transit in Mice.[a]

Dose (mg/kg)	Length of Small Intestine (cm)	Distance Traveled by Charcoal Meal (cm)	Peristaltic Index (%)	% Inhibition
Control	50.28 ± 3.05	38.98 ± 3.06	77.77 ± 5.29	—
80ME 100	53.84 ± 3.35	23.58 ± 5.75	43.87 ± 10.65	43.59
80ME 200	59.08 ± 2.66	12.00 ± 4.97[b]*	21.03 ± 8.64[b]*	72.96
80ME 400	56.96 ± 1.85	9.5 ± 4.03[b]*	16.01 ± 6.84[b]**	79.41
Loperamide 3	58.82 ± 2.75	8.6 ± 5.28[b]*	14.39 ± 8.88[b]**	81.5

[a]Values are given as mean ± standard error of the mean (n = 5); analysis was done using one-way analysis of variance followed by Tukey honestly significant difference (HSD) post hoc test.
[b]Compared with negative control values.
*$P < .01$, **$P < .001$.

Table 4. Effects of 80% Methanol Extract (80ME) of the Leaves of *Dodonaea viscosa* on Gastrointestinal Fluid Accumulation in Mice.

Dose (mg)	Volume of Intestinal Contents (mL)	% Inhibition	Weight of Intestinal Contents (g)	% Inhibition
Negative control	0.86 ± 0.12	—	1.19 ± 0.07	—
80ME 100	0.36 ± 0.05[b]**	58.14	0.58 ± 0.07[b]*	51.26
80ME 200	0.34 ± 0.04[b]**	60.47	0.52 ± 0.11[b]*	56.30
80ME 400	0.30 ± 0.03[b]**	65.12	0.51 ±0.14[b]*	57.14
Loperamide 3	0.26 ± 0.04[b]**	69.77	0.47 ±0.11[b]*	60.5

[a]Values are given as mean ± standard error of the mean (n = 5); analysis was done using one-way analysis of variance followed by Tukey honestly significant difference (HSD) post hoc test.
[b]Compared with negative control values.
*$P < .01$, **$P < .001$.

0.07, respectively. All the doses of the plant extracts were able to significantly inhibited castor oil–induced gastrointestinal fluid accumulation. Accordingly, the volume of intestinal contents for extract-treated groups at doses of 100, 200, and 400 mg/kg were 0.36 ± 0.05 ($P < .001$), 0.34 ± 0.04 ($P < .001$), and 0.30 ± 0.03 ($P < .001$), respectively. In addition, the plant extract was significantly decreased the weight of intestinal contents at 100 mg/kg (0.58 ± 0.07, $P < .01$), 200 mg/kg (0.52 ± 0.11, $P < .01$), and 400 mg/kg (0.51 ± 0.14, $P < .01$) as compared with the negative control group (Table 4).

In Vivo Antidiarrheal Index

ADI measures the combined effects of plant extract on purging frequency, the onset of diarrheal stool, and intestinal fluid accumulation. The ADI values of plant extracts were 62.55, 88.71, and 95.71 at doses of 100, 200, and 400 mg/kg, respectively. These results indicated that the plant extract produced dose-dependent antidiarrheal indices with the maximum effect at 400 mg/kg (Table 5).

Acute Oral Toxicity Test

The 80ME extract of the leaf of *D viscosa* did not produce any sign of toxicity or death during the observation periods of 14 days following oral administration of a single dose of 2000 mg/kg. The absence of mortality and signs of any toxicity up to 5 times the maximum effective dose of the extract demonstrated that 80ME has a broader safety margin and indicating that the

Table 5. In Vivo Antidiarrheal Indices of 80% Methanol (80ME) Extract of the Leaves of *Dodonaea viscosa*.

Dose (mg)	Delay in Defecation (Time of Onset in min, Dfreq) (%)	Gut Meal Travel Distance (Gmeq) (%)	Purging Frequency in Number of Wet Stool (%)	Antidiarrheal Index
Negative control	—	—	—	—
80ME 100	82.73	43.59	67.86	62.55
80ME 200	127.58	72.96	75	88.71
80ME 400	140.53	79.41	78.57	95.71
Loperamide 3	129.5	81.5	82.14	95.35

LD50 (median lethal dose) value of plant extract is greater than 2000 mg/kg in mice.

Discussion

People commonly use different parts of the plant for the treatment of various ailments, including diarrheal disease without any scientific basis about their safety and efficacy. However, various studies have been undertaken to validate the use of antidiarrheal activities of medicinal plants by investigating the biological activity of extracts of the plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water absorption, or reduce the intraluminal fluid accumulation.[47] Moreover, the study conducted on the alcohol and aqueous extracts of the roots of *D viscosa* demonstrated antidiarrheal activities against castor oil–induced diarrhea in reducing the frequency of defecation in mice.[20] However, root and whole-plant harvesting are more destructive to medicinal plants than collecting their leaves and flowers or buds.[48] Therefore, this study was aimed to evaluate the antidiarrheal effect of the 80ME leaf extract of *D viscosa* using Swiss albino mice models against castor oil–induced diarrhea, castor oil–induced gastrointestinal transit, and castor oil–induced gastrointestinal fluid accumulation as compared with the previous study.

Diarrheal disease is characterized by frequent defecation of feces of low consistency, which may be due to a disturbance in the transport of water and electrolytes in the intestines. Though there are multiple causes for diarrheal disease, the 4 major mechanisms behind the pathophysiology of diarrheas are (*a*) osmotic diarrhea, which is caused by increase in intraluminal osmolarity and decrease in water absorption; (*b*) secretory diarrhea, which increases the secretion of electrolyte; (*c*) deranged intestinal motility causing a decreased transit time[49]; and (*d*) inflammatory and infectious diarrhea, which is caused by disruption of the epithelium of the intestine due to bacterial, viral, or protozoal pathogens and the immune response to inflammatory conditions in the bowel.[50] In the management of diarrhea, antimotility and antisecretory agents are considered to be the mainstay agents used to decrease the pathophysiologic conditions responsible for the development of diarrhea.[51]

Castor oil has been substantially used for the induction of diarrhea in antidiarrheal studies because it releases ricinoleic acid, a metabolite that causes diarrhea.[52] Ricinoleic acid initiates diarrhea through mechanisms that include inflammation of gastrointestinal mucosa, leading to the discharge of prostaglandin, which stimulates gastrointestinal motility and electrolyte secretion, lowering electrolyte absorption from the intestine and colon, a mechanism that is much like the pathophysiologic techniques resulting in diarrhea.[53] Prostaglandins of the E series are well established to have diarrheagenic effects in experimental animals as well as in human beings. Therefore, the inhibitors of prostaglandin biosynthesis are considered to delay castor oil–induced diarrhea.[54] On the other hand, loperamide, the standard antidiarrheal drug used for the positive control is a synthetic opiate agonist activating the μ-opioid receptors in the myenteric plexus of the massive gut. Those receptors are positioned presynaptically on the endings of the parasympathetic cholinergic innervation of the intestinal smooth muscle, which exerts a facilitatory impact on clean muscle contractility.[55] Activation of μ-opioid receptors via loperamide inhibits the release of acetylcholine and subsequently relaxes smooth muscle tone inside the intestine wall.[56] These physiologic final results enhance phasic colonic segmentation and inhibit peristalsis, hence increasing intestinal transit time.[57] The inhibitory impact of loperamide on acetylcholine cause inhibition of secretion mediated with acetylcholine. As a result, loperamide reduces each day fecal volume, decreases fluid and electrolyte loss, and could increase stool viscosity and bulk density.[55]

Coming to the 80ME extract of the leaf of *D viscosa*, the findings of the study demonstrated that the plant extract was caused a significant delay in the onset of diarrhea, reduction in the frequency of wet fecal output and total fecal output, as well as decrease in the average weight of wet feces and total fecal output that were induced by castor oil. These findings are similar to the results reported from the hydroalcoholic extracts of the leaves of *Myrtus communis* at doses of 100, 200, and 400 mg/kg.[39] Besides, the percentage inhibition of defecation, weight of wet fecal output and the total weight of fecal output were observed in a dose-dependent manner, in which the highest percent of inhibition was observed at 400 mg/kg dose of the plant extract. This indicated that the higher dose of the plant extract is associated with a better antidiarrheal effect, which is comparable with the standard antidiarrheal drug loperamide. This could imply that the constituents of the plant extract, which are responsible for antidiarrheal activities are more likely to be concentrated in the higher doses of the plant extract or this might indicate that a relatively high dose of the plant extract is needed to produce a pronounced antidiarrheal effect. These findings are in agreement with reports from studies conducted on other species of plants.[39,58] Furthermore, these results are also in line with the findings of the previous study conducted on the root of *D viscosa* in terms of percentage protection of defecation.[20]

With regard to castor oil–induced gastrointestinal motility, the plant extract significantly ($P < .05$) inhibited the propulsive

movement of charcoal marker at the 2 higher test doses whereas the lower dose (ie, 100 mg/kg) of the plant extract was unable to produce significant inhibitory effect on the distance traveled by charcoal meal as compared with the negative control group. This could imply that the lower dose may have an insufficient concentration of active constituents responsible for the antimotility effect. This finding suggested that the plant extract has the antimotility effect at its higher doses, which opposed the effect of castor oil on the gastrointestinal motility of the mice in a similar manner to loperamide.[57] On the other hand, at all dose ranges used, the plant extract was able to significantly ($P < .001$) inhibit gastrointestinal fluid accumulation. In addition, the plant extract also revealed significant ($P < .01$) reduction in the weight of intestinal contents at all test doses used in the study. This might indicate that the plant extract has antisecretory effect, which reduced an excessive secretion mediated by irritant effects of ricinoleic acid, a metabolite of castor oil. It is widely reported that different antidiarrheal agents exert their effect through different mechanisms such as inhibiting secretion, decreasing motility, delaying intestinal transit, reducing intraluminal fluid accumulation or by enhancing water absorption.[59] Besides, the plant has also reported having anti-inflammatory and analgesic effects that could be related to inhibition of prostaglandin biosynthesis[28] and this in turns lead to inhibition of castor oil mediated diarrhea via inhibition of prostaglandin production. Additionally, the plant has also reported having antibacterial activities against some gram-positive and gram-negative bacteria, including those that are responsible for diarrheal diseases (eg, *E coli*, *Salmonella typhi*).[25] Though further studies are required, this might indicate a potential antidiarrheal activity of the plant extract against diarrhea induced by susceptible infective agents.

Moreover, the antidiarrheal activity of *D viscosa* leaf extract is also further strengthened by the *in vivo* ADI value of plant extract which measures how much the plant extract is effective in the management of diarrhea.[39] Another study also demonstrated that the higher the ADI value, the greater is the effectiveness of the extract in the treatment of diarrhea.[43] Accordingly, the ADI value increased in a dose-dependent manner in which the ADI of the higher dose of the plant extract is comparable to that of the standard drug loperamide. This could indicate that the plant has a potential antidiarrheal activity, which may serve as a template in the development of a novel antidiarrheal drug.

Furthermore, numerous types of the literature demonstrated that plants possessing alkaloids, flavonoids, saponins, steroids, and tannins had been reported to elicit antidiarrheal activity due to their antispasmodic impact on the gastrointestinal tract[60] and antisecretory properties.[61] Flavonoids have an ability to inhibit intestinal motility and hydroelectrolytic secretions whereas tannins precipitate proteins, reducing secretion and peristaltic movements.[62] In addition, tannin also causes muscle relaxation via decreasing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system.[63] Reports from literature also showed that tannins have an antispasmodic and muscle relaxant effect, flavonoids inhibit prostaglandin E2–induced intestinal secretion, saponins inhibit histamine release, terpenoids inhibit the release of prostaglandins, and phenols reduce intestinal secretion and transit and have an astringent action. All these actions lead to the inhibition of diarrhea by decreasing intestinal secretion and motility.[64] Most of these secondary metabolites were detected in the 80ME extract of the leaf of *D viscosa*. Though the specific mechanism of action of the plant has not yet established, the antidiarrheal activity of the plant extract may be produced by these chemical constituents, which contributed to its ability to delay in onset of diarrhea, antimotility, and antisecretory effects.

With regard to acute toxicity test, the plant extract was found to be safe as no sign of toxicity was observed in the acute oral toxicity test at the limit dose of 2000 mg/kg in mice. At the test dose, mortality and delayed toxicity were not observed in the 14 days posttreatment period. Based on the findings of the oral acute toxicity test, the LD50 value of the 80ME extract of the leaf of the plant is above 2000 mg/kg. Generally, if the LD50 value of the test chemical is more than 3 times of its minimum effective dose, the substance is considered as a good candidate for further investigation.[65] Therefore, the finding implies that the LD50 value of plant extract is more than three times of its minimum effective (100 mg/kg) dose, and the plant is a good candidate for further investigation. Overall, the finding of oral acute toxicity test indicated that the 80ME extract of the leaf of *D viscosa* is tolerable and safe after oral administration which validates the safe use of the plant in traditional settings.

Conclusion

The 80ME extract of the leaf of *D viscosa* showed antidiarrheal activity on evaluation in animal models using Swiss albino mice. The plant extract demonstrated a significant delay in the onset of diarrhea, reduced the frequency of wet feces and also endowed with significant antisecretory effects at all doses evaluated experimentally. In addition, the plant extracts also indicated the antimotility effect at its higher doses. Though further investigations are warranted using different antidiarrheal models and solvents, at this level the findings of the study confirmed the traditional claim of antidiarrheal activity of the plant. Moreover, the study also evaluated the acute toxicity of the plant extract in which the plant is found to be nontoxic and its LD50 is greater than 2000 mg/kg, which ensures the safe use of the plant extracts in folk medicine.

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Declaration of Conflicting Interests

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Ethical Approval

The animals were handled according to the guidelines for Care and Use of Laboratory Animals and OECD Guidelines. The study was approved by the Ethical Committee of the College of Health and Medical Sciences, Haramaya University before the actual experimental activities was started.

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