Evaluation of In vivo antidiarrheal activity of hydro-methanolic extract of the root of *Rumex nepalensis* in Swiss Albino mice

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**A B S T R A C T**

*Background:* Natural products have been utilized by human beings for thousands of years to relieve a variety of ailments, including diarrhea. Conventional antidiarrheal drugs are associated with multiple adverse effects and contraindications. Traditionally, *Rumex nepalensis* by crushing the root, mix with water and then drunk the juice is extensively used for treating diarrhea. However, no scientific research has been done yet to support its anti-diarrheal efficacy and safety. Hence, the aim of the study was to evaluate the antidiarrheal activity and safety profile of the plant in mice.

*Methods:* The hydro-methanolic extract was extracted through a cold maceration technique using 80% methanol. Castor oil-induced diarrheal, gastro-intestinal transit, and enteropooling models have been employed to assess the antidiarrheal activity of the test extract at doses of 100, 200, and 400 mg/kg.

*Results:* The crude root extract caused no mortality at a single limit test dose of 2 g/kg throughout the first 24 h and for the rest of the 14 days. In a castor oil-induced diarrheal model, the hydro-methanolic extract markedly delayed the onset of diarrhea, reduced the weight of wet and total feces at 100 (P<.05), 200 (P<.01), and 400 mg/kg (P<.001) test doses. Meanwhile, at 200 (P<.01) and 400 mg/kg (P<.001) doses, the plant extract considerably lowered the weight and volume of intestinal contents. In the gastro-intestinal transit model, however, a dramatic inhibition in the charcoal meal travel was noticed at 100 (P<.05), 200 (P<.01), and 400 mg/kg (P<.001) test doses. The peak antidiarrheal index was exhibited at the highest dose of the test extract.

*Conclusion:* The study speculated that *Rumex nepalensis* root extract possesses antidiarrheal activity, which could be owing to its inhibitory effect on both gastro-intestinal motility and fluid secretion.

1. Introduction

Diarrhea is defined as the passing of loose or watery stools at least three times per day. It is majorly classified as acute and chronic based on the length of time that symptoms last [1]. Diarrheal diseases have long been a serious public health concern in low-income nations, with high rates of morbidity and mortality. Globally, diarrhea is the second greatest cause of death in children under the age of five, accounting for 9% of all child deaths [2]. Under 5 years of age, it is a cause of about 15% or more than 1600 child deaths on a daily basis of all child deaths [3]. Roughly, 80% of all diarrheal deaths in children occur in African and Southeast Asian countries [4]. About 25% and 31% of the overall diarrheal burden has been attributed to diarrhea among children below the age of five in Africa and Asia, respectively, with the highest death rate in Africa [5].

Natural products have been utilized by human beings for thousands of years to heal from a variety of ailments. In impoverished nations, traditional medicines are virtually entirely used by the majority of people to treat various types of ailments, including diarrhea [6]. Medicinal plants have been shown to be a rich supply of biologically active chemicals, with many of them being used to develop lead compounds [7]. Around 25% of conventional medicines are derived straight from herbal products. The others are synthetic equivalents obtained from prototype molecules made from medicinal plants [8,9]. “Berberine”, for example, is synthesized from the root and bark extract of *Berberis aristata* and is currently used to treat diarrhea in modern pharmacopoeia [10].

In Ethiopian folkloric medicine, a variety of medicinal herbs with
antidiarrheal effects have been frequently used by human beings. Among these, the leaves of Cordia africana [11], the seeds and leaves of Ruta chalepensis, the root of Solanum incanum L. [12], the seed of Linum usitatissimum L., the root of Salvia nilotica [13], the leaf of Verbena Officinalis L., Vernonia amygdalina, and Withania somnifera (L.) [14], and the root of Rumex nepalensis Spreng are widely utilized. Nevertheless, a few of these medicinal plants, such as Rumex nepalensis, have not yet been thoroughly investigated for their healing potential and safety profile. Therefore, the aim of the study was to evaluate the antidiarrheal activity and safety profile of Rumex nepalensis in mice.

Rumex nepalensis Spreng belongs to the Polygonaceae, popularly known as the buckwheat family. The family comprises over 1200 species in 52 genera. It has various local names: “Yeshewa Tul” in Amharic, “Shuulltii” in Oromifa, and “Shembobaeta” in Tigirigna [15]. Rumex is a genus with over 250 species found all over the world, including Europe, Asia, Africa, and America. It is also widely dispersed throughout Ethiopia, including Tigray, Amhara, Oromia, and the southern regions [16].

In Ethiopia, the plant is traditionally utilized for a wide range of human diseases such as stomach aches, rheumatism, tonsillitis, ascariasis, uterine bleeding, abdominal bleeding, diarrhea, toothache, acute febrile illness, abdominal pain, dysentery, and oral ulcer [17]. Various scientific studies have been reported to have anti-oxidant, anti-fungal, anti-bacterial, anti-histaminic, anti-cholinergic, anti-bradykinin, anti-prostaglandin, anti-pyretic, anti-inflammatory, insecticidal, analgesic, and central nervous system depressant activities [18].

Despite the fact that a huge array of pharmaceutical drugs are accessible for diarrhea management, the majority of them are associated with adverse effects and contraindications [19]. Human beings in different parts of Ethiopia have long been employing a variety of herbal preparations with antidiarrheal properties. Among those, Rumex nepalensis, by crushing the root, mixing with water and drinking the juice, is extensively used [20–22]. However, no scientific research has been done to support its antidiarrheal properties. It is far more important to properly assess the therapeutic uses of these medicinal plants to develop new antidiarrheal medications with innovative mechanisms of action and minimal untoward effects. As a result, the scientific evidence for Rumex nepalensis’s antidiarrheal effect must be validated.

Plant species from the same genus “Rumex”, R. nervosus and R. hastatus, have substantial antidiarrheal properties [23,24]. Therefore, R. nepalensis, which belongs to the same genus, could have a similar effect. Antidiarrheal action has also been reported in herbal products containing alkaloids, tannins, saponins, flavonoids, and terpenoids [25]. Previous phytochemical studies have shown that R. nepalensis possesses such secondary metabolites, implying that the plant may have antidiarrheal activity [15].

Literatures has shown that oxidative stress and inflammation are involved in the pathogenesis of diarrhea [26,27]. Interestingly, the root extracts of R. nepalensis possess strong in vivo anti-inflammatory and in vitro anti-oxidant activity [28]. Therefore, numerous traditional claims for diarrhea and prior tests might be used as a support to check further in vivo antidiarrheal activity.

2. Materials and methods

2.1. Drugs and chemicals reagents

The study included the following drugs and chemicals: Absolute methanol (Nice Chemicals Pvt Ltd, India), atropine sulfate (Acultife, Health Care Pvt Ltd, India), activated charcoal (SD Fine Chem Ltd, India), loperamide hydrochloride (Remedica, Cyprus), castor oil (Amman Pharmaceutical, Jordan), Wagner’s reagent (Research-Lab Fine Chem Industries, India), lead acetate 1% (Guangdong Guanghua chemicals factories, China), sodium hydroxide (BDH, chemical lab, England), ferric chloride (BDH Laboratory Supplies Poole, England), chloroform (Hi-Media Laboratory Reagents, India), and sulfuric acid (HiMedia Laboratories Pvt. Ltd., India). All drugs and chemical reagents used in this study were analytical grade and stemmed from public and private sources.

2.2. Experimental animals

The experiment was carried out on healthy Swiss albino mice of either sex, weighing 25–30 g and aged 6–8 weeks. The animals were sourced from the Ethiopian Public Health Institute (EPHI). The mice were housed in plastic cages with softwood shavings and chips as bedding at favorable environmental conditions (25 ± 2 °C, 12:12 h light/dark cycle), with unlimited access to standard pellet and water ad libitum. They were given seven days to acclimatize the laboratory environment before initiating the main experiment. All protocols used in this study followed the guiding standards for animal research, as established by the guiding principles for animal care and use [29].

2.3. Collection of plant material and identification

In December 2021, fresh R. nepalensis roots were gathered from the environs of Tara Gedam, which is located in the Libo Kemkem district in the South Gondar Zone of Amhara Regional State, North West Ethiopia, 637 km from Addis Ababa, the capital city of Ethiopia. Botanical identification of the plant sample was verified by a botanist at the Department of Biology, College of Natural and Computational Science, Debre Tabor University, Debre Tabor, Ethiopia, with the code number AY04 and then put there for future reference. The roots of the plant were then collected in bulk, carefully rinsed with cold water, and left to dry for two weeks at ambient temperature. The roots were then mechanically ground into coarse powder and kept in plastic container until required for extraction.

2.4. Extraction of plant material

The dried root powder of Rumex nepalensis was extracted through the cold maceration technique using 80% methanol. With three Erlenmeyer conical flasks, 400 g of powder was soaked in a total of 5000 ml of 80% methanol and then shaken for 72 h using a mini orbital shaker. The extract was then first filtered with gauze (muslin cloth) on the third day, followed by Whatman filter paper No. 1. To maximize the product, the remaining residue was re-soaked twice in similar solvent and under similar circumstances for six days. The supernatant from each subsequent filtration was then collected and concentrated on a rotary evaporator at 40 °C to remove methanol. Thereafter, the concentrated filtrates were stored in a deep freezer overnight followed by drying with a lyophilizer calibrated at –50 °C to remove water. Finally, the extraction yield was computed, marked, and placed in an airtight container under –4 °C in a refrigerator until used for the study [15].

2.5. Preliminary phytochemical screening assay

The existence or absence of bioactive phytochemicals such as alkaloids, phenols, flavonoids, glycosides, steroids, saponins, terpenoids, tannins, and anthraquinones was determined using a standard qualitative approach [30].

2.6. Quantitative estimation of phytochemicals

2.6.1. Estimation of flavonoid content

The following standard procedure was used to determine the flavonoid content in the test extract [31]. The dried sample extract (2.50 g) was mixed with exactly 50 ml of 80% methanol in a 250 ml beaker, covered, and left to stand at 25 °C for 24 h. Then, shortly after the supernatant was discarded, the residue was extracted three times with the same amount of ethanol and filtered by using Whatman filter paper No.
42 (125 mm). The filtrates from each sample were then poured into a beaker and dried in a water bath. The dried filtrate was then cooled and weighed with a desiccator until a consistent weight was obtained. Lastly, the amount of flavonoids was estimated in percentage by using the following formula;

\[
\text{Flavonoid content (\%) = \frac{\text{Weight of flavonoid}}{\text{Weight of sample}} \times 100}
\]  

(1)

2.6.2. Estimation of phenolic content

The phenolic content of the test extract was assessed using a Folin reagent Ciocanteu’s microplate assay procedure with minor modifications. The quantity of phenols in the extract was then determined and articulated in gallic acid equivalent per gram of extract using a standard curve constituted with gallic acid [32].

2.6.3. Estimation of alkaloid content

The alkaloid content was measured by using the following technique as outlined by Ezeonu and Ejikeme [31]. Accordingly, 2.5 g of powdered sample extract was soaked into ethanol with 200 mL of 10% acetic acid in a 250 mL beaker and allowed to settle for 4 h. Shortly after filtration, the sample extract was then concentrated to one-quarter of its baseline volume in a water bath. After that, 15 drops of concentrated ammonium hydroxide were gradually added to the extract until complete precipitation was noticed. After 3 h of precipitation, the filtered liquid was then discarded, and the remnants were rinsed thoroughly with 20 mL of 0.1 M ammonium hydroxide and filtered using Gem filter paper (12.5 cm). Then, the residue was concentrated in a hot oven set at 40 °C, and the weight was estimated using an electronic balance. Subsequently, the content of alkaloids was calculated and articulated in percentages using this equation;

\[
\text{Alkaloid content (\%) = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100}
\]  

(2)

2.6.4. Estimation of tannin content

The tannin content in the dried sample extract was determined by using the Toma et al. method with little modification [32]. The dried sample extract (1 g) was taken and diluted in 1 mL of 80% ethanol. Following dilution, 50 µl of the sample solution was poured into 100 µl of vanillic acid solution (4% w/v) with 50 µl of concentrated HCl. The absorbance was then measured immediately at 500 nm and the quantity of tannin was computed using a calibration curve made from catechin as a standard. The results were presented in catechin equivalent (mg) per gram of the dried extract.

2.7. Acute oral toxicity study

The (OECD, 2008) 425 guidelines were used to undertake an acute oral toxicity assay in normal, mature, and non-pregnant female Swiss Albino mice [33]. Accordingly, five female mice weighing 25–30 g were employed. All animals were deprived of food for 3 h before being loaded with the plant extract. After being fasted, one female mouse was administered a single limited test dose of 2 g/kg of the test extract orally. The mouse was then extensively followed for 4 h, every 30 min, for any signs of toxicity and mortality within the first 24 h. Based on the findings of the first mouse, the next four mice were delivered an equivalent dose of the extract in a sequential order. The mice were then kept individually and monitored consistently for 4 h with a 30 min gap and then daily for two weeks for any signs of toxicity such as behavioral, autonomic, neurologic, and physical anomalies.

2.8. Grouping and dosing of animals

For all models, thirty mice were employed. The mice were randomly divided into five groups, each consisting of six animals. Group I was assigned as negative control and administered with distilled water (10 mL/kg), while groups II, III, and IV were assigned as tested groups and given lowest dose (100), middle dose (200), and higher dose (400 mg/kg) root extract of R. nepalensis, respectively. Group V was served as a positive control, receiving the standard drug (loperamide 3 mg/kg for castor oil-induced diarrhea and anti-enteropooling tests; atropine 3 mg/kg for anti-motility tests). All the given treatments were given once, and the dose was calculated based on the acute toxicity study results and pilot study.

2.9. Models and investigation of antidiarrheal effect

2.9.1. Castor oil-induced diarrhea in mice

The technique proposed by Tefere et al. was applied in this study with minimal modification [34]. Thirty mice were fasted for 18 h and then divided into five groups, each with six mice, and treated as stated in the grouping and dosing section. All the given treatments were administered through the oral route. Each mouse was given 0.5 mL of castor oil 1 h after treatment to induce diarrhea. The animals were then placed separately in a plastic cage with a white clean paper floor. Each time the mouse defecated, the floor lining was replaced. After that, the onset of defecation, total number of fecal output, and consistency of feces voided by the mice were all recorded during the 4 h follow-up period and compared with negative control. The results were presented as percentage of diarrheal inhibition and weight of fecal output, and the following formula was used:

\[
\% \text{Inhibition of diarrhea} = \frac{\text{Mean number of wet feces (negative control − test)}}{\text{Mean number of wet feces of negative control}} \times 100
\]  

(3)

\[
\text{Percentage of wet fecal output} = \frac{\text{Mean weight of wet feces of each group}}{\text{Mean weight of wet feces of control}} \times 100
\]  

(4)

\[
\text{Percentage of total fecal output} = \frac{\text{Mean weight of total feces of each group}}{\text{Mean weight of total feces of control}} \times 100
\]  

(5)

2.9.2. Castor oil-induced enteropooling in mice

Using the method outlined by Ezeja and Ezeigbo, the ability of the crude extract to suppress intestinal fluid accumulation was assessed [35]. Thirty mice were arbitrarily chosen and fasted for 18 h. Immediately before the experiment, the mice were assigned into five groups of six animals each and dosed as previously described. All mice were then given castor oil (0.5 ml) 1 h later. After 1 h of giving castor oil, the mice were killed, and the small intestine of each mouse from the pylorus to the cecum was dissected out and weighed immediately. Afterwards,
each mouse’s intestinal content was collected by milking, and the volume was recorded. The small intestine was then reweighed and the difference between the full and empty intestine was computed. Later, the following equation was used to calculate the percentage inhibition of the volume and weight of intestinal contents.

\[
\text{% inhibition (VIC)} = \frac{\text{Mean volume of intestinal fluid (negative control)} - \text{Mean volume of intestinal fluid of test}}{\text{Mean volume of intestinal fluid of negative control}} \times 100
\]

Where, VIC = volume of intestinal content

\[
\text{% inhibition (WIC)} = \frac{\text{Mean weight of intestinal content (negative control) - Mean weight of intestinal content of test}}{\text{Mean weight of intestinal content of negative control}} \times 100
\]

Where, WIC = weight of intestinal content.

2.9.3. Gastro-intestinal motility test by charcoal meal

Thirty mice were chosen arbitrarily and deprived of food for 24 h before starting the procedure, but with free access to water. The mice were then randomly divided into five groups, each containing six mice, just before the experiment. Afterwards, the animals were treated as stated earlier, before 30 min of castor oil delivery. Each mouse was then given 0.5 ml of 10% charcoal suspension in distilled water orally after 30 min of being given castor oil. After 30 min of giving the marker, mice were sacrificed, the abdomen opened, and the small intestine was isolated. Later, the entire length of the small intestine and the distance travelled by the charcoal from the pylorus to the cecum were determined. Finally, the peristaltic index and percentage of inhibition were calculated using the formula given below [36].

\[
\% \text{Inhibition of motility} = \frac{\text{Distance travelled by the control} - \text{Distance travelled by the test group}}{\text{Distance travelled by the control}} \times 100
\]

Where, \( \mu c \): Mean distance travelled by the control, \( \mu t \): Mean distance travelled by the test group

Peristalsis index = \( \frac{\text{Mean Distance travelled by charcoal meal}}{\text{Mean length of small intestine}} \times 100 \) (9)

2.9.4. In vivo antidiarrheal index

The in vivo antidiarrheal index (ADI) of the positive control and the experimental group was calculated using the equation formulated by Aye-Than et al. [37].

\[
\text{In vivo antidiarrheal index} = \sqrt{Dfreq \times Gmeq \times Pfreq}
\]

Where, Dfreq = Delay in onset of defecation (in % of control) derived from castor oil diarrheal test; Gmeq = Gut meal travel reduction (in % of control) derived from charcoal meal test, and Pfreq = purging frequency as the number of wet stool reduction (in % of control) derived from castor oil-induced diarrheal test.

2.10. Data quality control

Data quality was ensured by utilizing a simple random sampling approach to assign experimental animals into groups, blinding the data collection system, adhering to and implementing standardized protocols, and using analytically graded materials.

2.11. Data analysis

The data were expressed as mean standard error of the mean (SEM), and statistical analysis was performed using the statistical package for social science software version 24. One way analysis of variance (ANOVA) was employed to analyze statistical differences between groups, followed by a post hoc Tukey’s test for multiple comparisons. The findings were declared statistically significant at a P value of less than 0.05.

3. Results

3.1. Extraction yields of crude extract

Out of 400 g of root powder, 72 g were obtained with a percentage yield of 18% at the end of maceration.

3.2. Preliminary phytochemical screening

As per a preliminary phytochemical screening test, alkaloids, sapo- ninis, tannins, flavonoids, terpenoids, phenols, and steroids were found in the crude root extract of *Rumex nepalensis*. Glycosides and anthraquinones, however, were absent (Table 1).

| Bioactive phytochemicals | Tests employed for screening | Result |
|--------------------------|-----------------------------|--------|
| Alkaloids                | Wagner’s test               | +      |
| Glycosides               | Glycoside test              | –      |
| Saponins                 | Honey comb test             | +      |
| Tannins                  | Lead acetate test           | +      |
| Flavonoids               | Shinoda test                | +      |
| Terpenoids               | Salkowski’s test            | +      |
| Phenols                  | Lead acetate test           | +      |
| Plant steroids           | Salkowski’s test            | +      |
| Anthraquinones           | Borntrager’s test           | –      |

Note: - (+): present, (-): absent.

Table 1
Preliminary phytochemical screening of hydro-methanolic root extract of *Rumex nepalensis*.

| Test extract | Phenolic content (mg/g) | Flavonoid content (g) | Tannin content (mg/g) | Alkaloid content (g) |
|--------------|-------------------------|-----------------------|-----------------------|----------------------|
| *Rumex nepalensis* | 81.45 (8.1%)          | 0.18 (7.2%)           | 51.32 (5.1%)          | 0.12 (4.8%)          |
the quantities were found to be 81.45 mg/g (8.1%), 0.18 g (7.2%), and 0.06 g (7.2%) of castor oil-induced diarrhea in mice.

3.4. Acute oral toxicity test

The hydro-methanolic root extract of *R. nepalensis* caused no mortality at a single limit test dose of 2 g/kg throughout the first 24 h and for the rest of 14 days, as per the study. Moreover, the toxicity study did not show any notable signs and symptoms of overt toxicity such as behavioral, psychiatric, somatic, or physical anomalies.

3.5. Effects of the hydro-methanolic extract on castor oil-induced diarrheal model

Relative to the negative control, the crude root extract of *R. nepalensis* significantly slowed (P < 0.05) the onset of diarrhea at both test doses (200 and 400 mg/kg), with a better effect at the maximum dose (P < 0.001). At this dose (400 mg/kg), the test extract exhibited a high degree of delay in diarrhea onset (63.7 ± 1.89), which is a bit comparable to the reference drug (67.6 ± 1.52). Likewise, the number of wet feces was found to be decreased with hydro-methanolic extract at three successive doses (100, 200, and 400 mg/kg) in a dose-dependent fashion. The percentages of inhibition defecation were 29.7%, 40.6%, and 50.7%, respectively. Similarly, loperamide 3 mg/kg (reference drug) has also drastically reduced (P < 0.001) the number of wet defecations with percentage inhibition (81.9%). Moreover, the effect of the test extract on the weight of wet feces was assessed via this model and the activity was found to be significant at 100 (P < 0.05), 200 (P < 0.01), and 400 mg/kg (P < 0.001). The percentage reduction in weight of wet fecal output was similarly diminished, with the greatest reduction at 400 mg/kg (29.6%). Nonetheless, the reduction was less compared to loperamide, which is 50% (Table 3).

### Table 3

| Group      | Onset of diarrhea (min) | No of wet feces | Total no of feces | Wt. of wet feces (g) | Wt. of total feces (g) | % inhib. of defecation | %WWFO | %WTFO |
|------------|--------------------------|-----------------|-------------------|----------------------|------------------------|------------------------|-------|-------|
| NC         | 43.6 ± 2.10              | 13.8 ± 1.01     | 15.7 ± 0.67       | 5.4 ± 0.30           | 5.8 ± 0.30             | –                      | –     | –     |
| RNRE100    | 45.9 ± 2.34              | 9.7 ± 1.41a*    | 14.0 ± 1.73       | 4.4 ± 0.18a*         | 4.3 ± 0.36a*           | 29.7%                  | 81.5% | 74.1% |
| RNRE200    | 56.8 ± 1.78a**           | 8.2 ± 0.60a*    | 11.2 ± 0.48a*     | 4.2 ± 0.17a**        | 3.4 ± 0.20a**          | 40.6%                  | 77.8% | 58.6% |
| RNRE400    | 63.7 ± 1.89a***          | 6.8 ± 0.70a***  | 9.5 ± 0.76a**     | 3.8 ± 0.10a***       | 3.1 ± 0.08a***         | 50.7%                  | 70.4% | 53.4% |
| LPR3       | 67.6 ± 1.52a***          | 2.5 ± 0.76a***  | 3.5 ± 0.76a***    | 2.7 ± 0.32a***       | 2.3 ± 0.12a***         | 81.9%                  | 50%   | 39.7% |

Values are expressed as mean ± SEM (n = 6) and analyzed using one way ANOVA followed by Post Hoc Tukey test. *Compared with negative control. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. NC = negative control, RNRE = *Rumex nepalensis* root extract, LPR3 = loperamide 3 mg/kg, ml = milliliter, and g = gram.

### Table 4

| Group      | Volume of Intestinal Content (ml) | % Inhibition | Weight of Intestinal Content (g) | % Inhibition |
|------------|-----------------------------------|--------------|----------------------------------|--------------|
| NC         | 2.3 ± 0.12                        | –            | 2.0 ± 0.09                       | –            |
| RNRE100    | 2.2 ± 0.12                        | 4.3%         | 1.8 ± 0.05                       | 10%          |
| RNRE200    | 1.5 ± 0.06a**                     | 34.8%        | 1.6 ± 0.06a*                     | 20%          |
| RNRE400    | 1.4 ± 0.19a*                      | 39.1%        | 1.5 ± 0.08a*                     | 25%          |
| LPR3       | 1.1 ± 0.16a***                    | 52.2%        | 1.4 ± 0.07a***                   | 30%          |

Values are expressed as mean ± SEM (n = 6) and analyzed using one way ANOVA followed by Post Hoc Tukey test. *Compared with negative control. \*P < 0.01, \*\*P < 0.001. NC = negative control, RNRE = *Rumex nepalensis* root extract, LPR3 = loperamide 3 mg/kg, ml = milliliter, and g = gram.

3.3. Quantitative estimation of bioactive chemicals

The total phenolic, flavonoid, tannin, and alkaloid contents of the crude root extract of *Rumex nepalensis* are summarized in Table 2. Thus, the quantities were found to be 81.45 mg/g (8.1%), 0.18 g (7.2%), and 0.12 g (4.8%), respectively.

### Table 5

| Group      | Length of small intestine (cm) | Distance travelled by charcoal meal (cm) | Peristaltic index (%) | % Inhibition |
|------------|--------------------------------|----------------------------------------|-----------------------|-------------|
| NC         | 63.2 ± 0.95                    | 52.3 ± 0.76                            | 82.9 ± 1.40           | –           |
| RNRE100    | 60.7 ± 0.88                    | 45.7 ± 0.88a*                          | 75.3 ± 1.03a*         | 12.6        |
| RNRE200    | 60.3 ± 0.88                    | 42.7 ± 1.23a**                         | 70.8 ± 1.05a*         | 18.4        |
| RNRE400    | 60.2 ± 0.98                    | 38.8 ± 1.25a**                         | 64.5 ± 1.15a***       | 25.8        |
| ATR3       | 57.7 ± 1.23a*                  | 35.3 ± 1.12a**                         | 61.2 ± 0.71a***       | 32.5        |

Values are expressed as mean ± SEM (n = 6) and analyzed using one way ANOVA followed by Post Hoc Tukey test. *Compared with negative control. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. NC = negative control, RNRE = *Rumex nepalensis* root extract, ATR3 = atropine 3 mg/kg, and cm = centimeter.

### Table 6

| Group      | Dfreq | Gmeq | Pfreq | ADI |
|------------|-------|------|-------|-----|
| NC         | –     | –    | 29.7  | 12.6|
| RNRE100    | 5.3   | 12.6 | 29.7  | 12.6|
| RNRE200    | 30.3  | 18.4 | 40.6  | 28.3|
| RNRE400    | 46.1  | 25.8 | 50.7  | 39.2|
| POC        | 55.1  | 32.5 | 81.9  | 52.7|

Note: NC = negative control, RNRE = *Rumex nepalensis* root extract, POC = positive control, Dfreq = Delay in defecation time; Gmeq = Gut meal travel reduction; Pfreq = Purging frequency or % reduction in number of wet stools, and ADI = antidiarrheal index.

3.6. Effects of the hydro-methanolic extract on castor oil-induced enteropooling

The findings of the effect of *R. nepalensis* extract on castor oil-induced enteropooling are tabulated as shown below. When compared to the negative control, studies on this model revealed that the test extract considerably reduced the intraluminal volume of fluid accumulation at 200 (P < 0.01) and 400 mg/kg (P < 0.01). Conversely, at a dose of 100 mg/kg, the activity of the test extract was not significant statistically. At the highest dose of the test extract, the maximum percentage inhibition of the volume of intestinal content was recorded (39.1%). Correspondingly, relative to the negative control, the test extract markedly reduced the weight of intestinal contents at 200 (P < 0.001) and 400 mg/kg (P < 0.001). At these respective doses, the percentage inhibition in the weight of intestinal content has been shown to be 20% and 25%, respectively. At the extract’s ceiling dose (400 mg/kg), a higher degree of reduction of the aforementioned parameter was observed (25%), which is comparable to the existing drug (30%) (Table 4). In contrast, the activity of the extract at 100 mg/kg was minimal relative to the negative control.
3.7. Effects of the hydro-methanolic extract on gastro-intestinal motility

The effect of the plant extract on charcoal meal transit time in mice is summarized below (Table 5). The findings revealed that all three respective doses of the extract (100, 200, and 400 mg/kg) substantially reduced the distance travelled by the marker in a dose-dependent manner. The peristalsis index of the marker in animals given 100 mg/kg of the extract was 75.3 ± 1.03 (P < .05), while in animals treated with 200 mg/kg of the extract was 70.8 ± 1.05 (P < .01). Whereas the group of mice given 400 mg/kg of the extract was 64.5 ± 1.15 (P < .001). When compared to the vehicle, the mean percent inhibition of the charcoal meal propulsion was decreased by 12.6%, 18.4%, and 25.8% in groups of animals given 100, 200, and 400 mg/kg of the crude extract, respectively. Relative to negative control, the maximal dose showed the highest degree of reduction in charcoal meal propulsion (25.8%), which is comparable to the conventional drug (32.5%). Compared to atropine, however, the test extract at the lowest and middle dose showed a smaller degree of reduction in intestinal motility of the charcoal meal.

3.8. Effect of the test extract on in vivo antidiarrheal index

The test extract’s in vivo antidiarrheal index (ADI) was calculated by combining three metrics (Table 6). At doses of 100, 200, and 400 mg/kg, the ADI values were 12.6, 28.3, and 39.2, respectively. Whereas the conventional drug produced a maximum ADI (52.7). These findings suggested that the plant extract had a dose-dependent antidiarrheal activity, with 400 mg/kg showing the highest effect.

4. Discussion

In different parts of Ethiopia, Rumex nepalensis is traditionally used by traditional healers for treating a variety of health problems, including diarrhea, despite the lack of scientific support for its safety and efficacy. As a result, the purpose of this investigation was to prove the antidiarrheal properties of Rumex nepalensis Spreng by using castor oil-induced diarrhea, castor oil-induced enteropooling, and charcoal meal anti-motility test models in mice. In this trial, a castor oil-induced diarrheal test was applied to verify the overall antidiarrheal activity of the crude extract. Whereas, anti-enteropooling and anti-motility models were adopted to try to come up with some of the possible mechanisms by which the natural substance showed antidiarrheal activity.

Using water as a solvent, the root of Rumex nepalensis is traditionally utilized orally to relieve diarrhea. In this investigation, however, hydro-methanolic was used. Because of their high polarity index, hydro-alcoholic solvent mixtures have been shown in studies to have a high extraction yield [38]. As methanol is hydrophilic in its nature, it may extract a broad array of chemicals with varying polarity using a hydro-methanolic solvent mixture. Hence, hydro-methanol has been employed as a solvent in this study for the initial extraction of the roots of R. nepalensis. Moreover, the solvent is not conducive to the growth of dangerous pathogenic microorganisms [39].

According to the acute toxicity test, during the 14-day follow-up period, neither death nor delayed signs and symptoms of acute toxicity were noticed at a single limit test dose of 2 g/kg of the crude extract. Thus, it is speculated that the medicinal plant has a wider safety margin, and the plant extract’s median lethal dose (LD50) is predicted to be above 2 g/kg. This finding was in agreement with past reports of acute oral toxicity results, which were conducted on the root extract of Rumex nepalensis [15]. This supports the non-toxic nature of the plant in its utilization in traditional settings.

In the present study, diarrhea was induced experimentally by using castor oil, as this model is the gold standard to examine the antidiarrheal effect and induces diarrhea in a similar manner to the natural pathophysiologic processes [40]. It is well known that the active constituent of castor oil, ricinoleic acid, is generated by the action of lipases on castor oil. Ricinoleic acid irritates and inflames the intestinal mucosa, causing the generation of prostaglandins, which leads to alterations in mucosal permeability, electrolyte transport, and intestinal peristalsis, resulting in hypersecretion and diarrhea [41]. Studies have shown that activated charcoal effectively adsorbs chemical substances, including drugs, on the surface of the intestine, thereby impeding absorption [42]. Therefore, a charcoal meal study was employed to explore the effect of R. nepalensis on peristaltic movement.

Compared with the negative control, the hydro-methanolic root extract of Rumex nepalensis had shown a substantial difference (P < .05) in the castor oil-induced diarrhea model at all test doses in all parameters. The onset of diarrhea, the number of wet and total stools, and the weight of wet and total feces were investigated as the primary indicator. In this model, the extract showed a dose-dependent effect, implying the maximal antidiarrheal activity is noted at the highest dose (400 mg/kg), with a percentage inhibition of defecation of 50.7%. This was in consonance with other reports, where methanolic root extract of Clusia abyssinica had shown a peak antidiarrheal effect at the maximal dose [43].

It is a fact that castor oil triggers diarrhea through hindering absorption and enhancing secretion and motility [44]. Thus, the antidiarrheal activity of R. nepalensis could be attributed to the presence of a variety of bioactive phytochemicals that enhance fluid and electrolyte absorption while inhibiting secretion and motility. In addition, the inducer triggers diarrhea by generating oxidative stress in the intestinal epithelium, which in turn disrupts the transport of electrolytes and water across the intestinal mucosa. It is scientifically authenticated that the root of R. nepalensis has in vitro anti-oxidant activity, which may account for its antidiarrheal activity [28].

In a castor oil-induced diarrhea model, scientific literature reported that non-steroidal anti-inflammatory drugs were found to delay the initiation of diarrhea [45]. Similarly, Rumex nepalensis root extract has been proven to show this effect, which could be owing to its anti-inflammatory properties [28]. The root extract of R. nepalensis was previously studied and found to have an inhibitory effect on cyclo-oxgenase, a key enzyme accountable for prostaglandin synthesis [39], which could be the other possible mechanism for its antidiarrheal effect. Prostaglandin promotes the secretion of intestinal fluid and electrolytes while diminishing glucose absorption, thereby causing diarrhea [46]. Through stimulation of intestinal secretion and motility, castor oil causes and stimulates diarrhea by nitric oxide [47]. Thus, the test extract’s antidiarrheal activity could also be attributed to inhibition of nitric oxide synthesis.

To substantiate the antidiarrheal activity of the plant extract of Rumex nepalensis, the probable mechanism of action was examined by using intestinal enteropooling and anti-motility models. In the castor oil-induced enteropooling model, relative to the negative control, it was noticed that the test extract dramatically reduced both the volume and weight of intestinal content at 200 and 400 mg/kg doses, in a dose-dependent manner. At maximal dose (400 mg/kg), the percentage inhibition of the weight of intestinal content (25%) was almost practically comparable to the reference drug (30%). This finding indicated that the plant extract had an anti-secretory effect, which could be one possible mechanism of action for its antidiarrheal activity. The findings are consistent with other studies in which the methanolic leaf extract of Osyris quadripartite has been shown to have a notable reduction in intestinal fluid accumulation [34]. As a result of this finding, the plant appears to be a good option for developing novel drugs with antidiarrheal activity.

The capability of the test extract to reduce intestinal secretion and/or increase water and electrolyte absorption may possibly account for this notable activity. Secretory diarrhea is coupled to chloride channel activation, which causes chloride outflow from the cell, resulting in excessive water secretion into the intestinal lumen and causing watery diarrhea [48]. Therefore, the anti-secretory effect of the hydro-methanolic root extract of R. nepalensis might be mediated by impeding this water secretion cascade into the gastric lumen.
In the castor oil-induced anti-motility test, the test extract markedly prolongs the intestinal transit at all test doses relative to the vehicle, notably the effect of the maximum dose being closer to the effect of atropine. This finding suggested that the crude extract hinders the peristaltic movement of the intestine, implying that the test extract had anti-motility activity. Concerning this, numerous plants have been found to have antidiarrheal activities by suppressing the castor oil-induced gastro-intestinal motility and its secretion [49].

Cholinergic activation increases gastro-intestinal motility, which causes diarrhea, and anticholinergics like atropine are clinically used to prevent diarrhea [50]. Therefore, the plant extract’s anti-motility activity was deduced to be attributable to its anti-cholinergic activity on intestinal mucosa. The stimulation of serotonergic receptors by physiological serotonin has been shown to enhance gut motility and intestinal oxide generation, which is prompted by ricinoleic acid, resulting in autacoid and prostaglandins [54]. Likewise, terpenoids suppress nitric oxide and electrolyte secretions by interfering with the activity of antidiarrheal effects [53].

Chemical components that have been shown to have a broad range of antidiarrheal effects [52]. For herbal products, terpenoids, tannins, and saponins. Medicinal plants bearing tannins, alkaloids, steroids, phenols, flavonoids, terpenoids, and saponins have been shown to have antidiarrheal effects [52]. For herbal products, tannins, saponins, terpenoids, and flavonoids are clearly the main chemical components that have been shown to have a broad range of activities, including antidiarrheal effects due to their anti-motility and anti-secretory effects [53].

Flavonoids and terpenoids are thought to impair intestinal motility, water and electrolyte secretions by interfering with the activity of autacoid and prostaglandins [54]. Likewise, terpenoids suppress nitric oxide generation, which is prompted by ricinoleic acid, resulting in decreased secretion in the intestinal mucosa [38]. Tannins are known for their antidiarrheal properties by denaturing proteins in the intestinal mucosa by forming protein tannate, which makes them more robust to chemical changes and thus reduces secretion [55]. Tannins also have an anti-motility activity due to their anti-spasmodic effect, which inhibits intestinal motility and secretions by decreasing Ca²⁺ influx or boosting calcium outflow [56]. Interestingly, all the aforementioned phytochemical constituents were found in the root of *Rumex nepalensis*, as per phytochemical studies. Therefore, these bioactive secondary metabolites might be accountable for the in vivo antidiarrheal activity of the hydro-methanolic root extract of the tested plant.

In general, ADI is a metric for determining how successful an extract is in treating diarrhea [36]. The greater the ADI value, the more successful in the treatment of diarrhea. The antidiarrheal index of *R. nepalensis* root extract was dose-dependent. Compared to the other doses, the highest test dose of the extract with the greatest ADI value has superior antidiarrheal activity.

5. Conclusions

The results of the study speculated that *Rumex nepalensis* root extract possesses antidiarrheal activity, which could be owing to its inhibitory effect on both fluid secretion and gastro-intestinal motility. The secondary metabolites detected could be responsible for the plant’s anti-diarrheal activity through a variety of mechanisms of action. However, more research is needed to determine the extract’s specific mechanism of action and to isolate and identify the active ingredient.

Ethical approval

Ethical approval was obtained from the Research and Ethics Committee of the College of Medicine and Health Sciences, Debre Tabor University, with code number SOP 2/109/14 to undertake the study. Throughout the study, animals were treated in compliance with international protocols for the care and use of laboratory animals.

Data availability

The datasets used in this investigation can be given by the corresponding author upon reasonable request.

Declaration of competing interest

The authors confirmed that they have no conflicts of interest.

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CRediT authorship contribution statement

**Yared Andargie:** Conceptualization, Methodology, Writing – original draft. **Woretaw Sisay:** Data curation, Supervision, Software. **Mulugeta Molla:** Project administration, Visualization, Validation. **Mulukzen Adela:** Investigation, Formal analysis, Resource provision.

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| RN           | *Rumex nepalensis* |
| ADI          | Antidiarrheal index |
| COX          | Cyclooxygenase |
| GI           | Gastro-intestinal |
| HT           | Hydro-tyramine |

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