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Evaluation of the antiulcerogenic activity of hydromethanol extracts of *Solanum incanum* L. (Solanaceae) leaves and roots in mice; single and repeated dose study

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ABSTRACT

Objective: The aim of this study was to evaluate the antiulcer activity of hydromethanol extracts of *Solanum incanum* L. (Solanaceae) leaves and roots in mice.
Methods: The antiulcerogenic activity of the plant extracts were evaluated using Pylorus ligation and ethanol induced gastric ulcers in fasted mice. Data were analyzed using one way ANOVA, and P-values <0.05 were considered statistically significant.
Result:Pylorus ligation-induced ulcer: Single dose and repeated daily dose administration of the leaf and root extracts for 10 days didn't significantly ($P > 0.05$) affect pH, total acidity and volume of gastric secretion. Single dose of both extracts significantly reduced ulcer score ($P = 0.036$) and ulcer index (leaf, $P = 0.037$; root, $P = 0.041$) at the dose of 400 mg/kg. Similarly, significant reduction in ulcer score was observed after repeated daily treatment with 200 mg/kg ($P = 0.030$) and 400 mg/kg ($P = 0.005$) of the leaf extract and 400 mg/kg ($P = 0.005$) of the root extract. In addition, repeated administration of 400 mg/kg of the leaf ($P = 0.004$) and root ($P = 0.005$) extracts significantly reduced ulcer index.
Ethanol-induced ulcer: Single dose of both extracts significantly reduced ulcer score at the dose of 200 mg/kg (leaf, $P = 0.017$; root, $P = 0.036$) and 400 mg/kg (leaf, $P = 0.001$; root, $P = 0.001$). Similarly, 200 mg/kg (leaf, $P = 0.002$; root, $P = 0.018$) and 400 mg/kg (leaf, $P = 0.001$; root, $P = 0.001$) of the extracts significantly reduced ulcer index after single dose treatment. Repeated daily treatment with leaf and root extracts for ten days caused a significant ($P = 0.037$, 0.001 and 0.001 for 100, 200 and 400 mg/kg leaf extract; $P = 0.026$, 0.018 and 0.001 for 100, 200 and 400 mg/kg root extract, respectively) reduction in ulcer score. In addition, both extracts significantly ($P = 0.041$, 0.004 and 0.000 for 100, 200 and 400 mg/kg leaf extract; $P = 0.038$, 0.008 and 0.000 for 100, 200 and 400 mg/kg root extract, respectively) reduced ulcer index after 10 days of treatment.
Conclusion: This study has revealed hydromethanol extracts of *Solanum incanum* leaves and roots have antiulcerogenic activity using *in vivo* models. The antiulcer activity of the plant is not related to acid anti-secretory action, suggesting the plant may have cytoprotective effect on the gastric mucosa.

1. Background

Peptic ulcer disease (PUD) is one of the most common gastrointestinal disorders in the world. Globally, PUD affects about 10% of the world population due to persistent *H. Pylori* epidemics in developing countries and an increased use of nonsteroidal anti-inflammatory drugs [1,2].

Conventional drugs currently used in the treatment of PUD (antacids,

Authors' contributions

All authors made substantial contributions to conception and design, analysis and interpretation of data; took part in drafting the article and revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Abbreviations

ANOVA	Analysis Of Variance
GIT	Gastro-Intestinal Tract
H. pylori	*Helicobacter pylori*
NSAIDs	Non-Steroidal Anti Inflammatory Drugs
PPIs	Proton Pump Inhibitors
PUD	Peptic Ulcer Disease
SILE	*Solanum incanum* leaf extract
SIRE	*Solanum incanum* root extract
UI	ulcer index
US	Ulcer Score

PPIs, H_2 antagonists, anticholinergic, mucosal protective agents and antimicrobials for *H. pylori* induced PUD) have no long term curative effect, relapse is usually common long after treatment. Moreover, these conventional drugs produce adverse reactions [3,4]. They also produce significant drug-drug interactions that limit their clinical use [5,6].

In Ethiopia, traditional medicine is an indigenous health care delivery system for 80% of the population, and more than 95% of traditional medicinal preparations originate from plant sources [7]. Previous studies have showed a large number of medicinal plants and their phytochemicals possess antiulcer activity [8–12]. Thus, medicinal plants may serve as a useful source of new medications for PUD. .

Solanu incanum L. (Solanaceae) is traditionally used for the treatment of PUD in Ethiopia. An ethnobotanical survey which was conducted around Atsbi and Adi Keyih, tigray region, Northern Ethiopia reported that both the leaf as well as root juice of *Solanum incanum* L. (Solanaceae) are taken orally to treat PUD [13]. However; the antiulcer activity of this medicinal plant has not been evaluated scientifically.

S. paniculatum and *S. nigrum*, similar plant species from the genus *Solanum*, have significant antiulcer activity [14,15]. As a result, *S. incanum* may have similar effect as it belongs to the same genus. Additionally, the antiulcer activity of medicinal plants is mainly due to the presence of phenolic compounds, flavonoids and saponins [15–17]. Previous phytochemical studies indicated that *S. incanum* contain these secondary metabolites known to have antiulcer activity [18–20] indicating the plant may have antiulcer activity.

It is known that oxidative stress plays an important role in the pathogenesis of PUD [21,22]. The protective effect of plant derived antioxidants against PUD has been explained in experimental studies which have demonstrated that antioxidants from plants are helpful in treating PUD through prevention of oxidative stress [16,17]. Interestingly, the root, leaf and fruit extracts of *S. incanum* possess strong *in-vitro* antioxidant activity [18,23]. As a result, this medicinal plant may have antiulcer activity.

Accordingly, the present study aimed at evaluation of the effects of hydromethanol extracts of *Solanum incanum* L. (Solanaceae) leaves and roots on pylorus ligation and ethanol induced gastric ulcer in mice.

2. Methods

2.1. Drugs, chemicals and instruments used in the study

Ethanol absolute (Indenta Chemicals, Mumbai, India), Ranitidine (Cadila, Addis ababa, Ethiopia), phenolphthalein indicator (Fine Chemicals, Mumbai, India), sodium hydroxide (Rankem, Mumbai, India), Methanol absolute (Nice chemicals, India), Analytical balance, pH meter, Distilled water, and Whatman filter paper No.1 were used. Analytical grade chemicals were used for the experiment.

2.2. Collection of the plant materials

Fresh roots and leaves of *Solanum incanum* L. were collected from the wild in Anbessamie town, northwest Ethiopia in December 2018, and getting a permission from Dera woreda environmental protection office was not necessary because the required sample was too small. Taxonomic identification of the plant was done by a botanist (Dr. Getinet Masresha), and a sample of the plant material was preserved in the Herbarium of University of Gondar for future reference (Voucher number, GM005/2011).

2.3. Preparation of the crude extracts through maceration

The leaves and roots of the plant were washed with distilled water and then allowed to dry under shade with optimal ventilation. Then, the dried leaves (125 g) and roots (220 g) were grinded separately and the coarse powder of the plant materials were macerated in 80% methanol for three days (72 h) in three successive volumes. Then the extracts were filtered through whatman filter paper No.1. The collected filtrates were dried in hot air oven at 39 °C. Finally, the dried leaf and root extracts were kept separately in desiccators until used for the study.

2.4. Laboratory animals

Albino mice (30–35 g) of either sex were used in the study. Mice were obtained from the animal breading house of pharmacy department, Wollo University. The animals were maintained in the animal house of pharmacy department, Wollo University under standard conditions (12 h of light and dark cycle) until they were used for the experiment. The animals were allowed free access to standard pellet laboratory diet and water *ad libitum*. Additionally, they were allowed to acclimatize the laboratory conditions for one week before used in the study.

2.5. Preliminary phytochemical analysis of Solanum incanum root and leaf extracts

Qualitative preliminary phytochemical screening tests were carried out for *Solanum incanum* root and leaf extracts as per the standard methods described by Tiwari P. et al., 2011 [24] and Pandey A. et al., 2014 [25] to indicate the presence or absence of alkaloids (Mayer's Test), phenols (Ferric Chloride Test), flavonoids (Alkaline Reagent Test), tannins (Gelatin Test), saponins (Froth Test), terpenoids (Copper acetate Test), glycosides (Legal's Test), steroids (Liebermann Burchard test) and anthraquinones (Borntrager's reaction for free anthraquinones).

2.6. Total phenolic content determination of Solanum incanum root and leaf extracts

The phenolic content of the extracts was determined using a Folin-Ciocalteu 96-well microplate assay method as explained by Zhang et al. [26]. The total phenolic content was calculated and presented as Gallic Acid Equivalent per gram of extract using a standard curve prepared by using gallic acid.

Total flavonoid content determination of Solanum incanum root and leaf extracts The flavonoid content was determined using the method explained by Ordonez et al. [27]. A volume of 0.5 ml of 2% Aluminum Chloride in ethanol solution was added to 0.5 ml of sample solution (1 mg/ml). After 1 h at room temperature, the absorbance was measured at a wave length of 430 nm. Total flavonoid content was calculated using the linear equation of a standard curve prepared with quercetin and expressed as mg quercetin equivalent per gram of crude extract.

2.7. Acute oral toxicity study of the extracts

Toxicity test was done based on the limit test recommendations of OECD No 425 Guideline [28]. On the first day of the test one female

Swiss albino mouse fasted for 3–4 h was given 2000 mg/kg of the plant extract orally. Then the mouse was strictly observed for physical or behavioral changes for the next 24 h, with special attention during the first 4 h. On the second day, other four female mice fasted for 3–4 h were recruited and administered a single dose of 2000 mg/kg of the extract and then observed in the same manner. The observation was continued for a total of two weeks for any signs of overt toxicity [28].

2.8. Experimental design

Animals were randomly assigned to different groups (8 groups for each ulcer model) each consisting of six animals (Fig. 1). All treatments were given orally 1 h before induction of experimental ulcer by using an oral gavage. Doses (100 mg/kg, 200 mg/kg and 400 mg/kg) of the plant extracts were determined based on the acute toxicity studies as per OECD guideline. All the doses of the plant extracts were dissolved in distilled water for administration at a volume not greater than 10 ml/kg body weight of mice. All drugs, extracts and ethanol were administered by oral route using oral gavage.

One h after ethanol administration, animals were sacrifice to measure Ulcer score and Ulcer index.

Four h after pylorus ligation, animals were sacrifice to measure Ulcer score, ulcer index and gastric secretion volume, PH and acidity.

Ethanol was administered (Ethanol induced ulcer) or Pylorus ligation was done (pylorus ligation-induced ulcer) 1 h after the 10th day treatment.

Ethanol was administered (Ethanol induced ulcer) or Pylorus ligation was done (pylorus ligation-induced ulcer) 1 h after treatment administration.

2.8.1. Repeated dose study

Treatments were administered daily for 9 days, and then animals were fasted for 24 h before receiving the 10th day treatment.

Animals were randomly assigned to 8 groups.

2.9. Pyloric ligation-induced ulcer model

2.9.1. Single-graded dose study

Forty eight fasted (for 24 h) male Swiss albino mice were randomly assigned to eight different groups (1–8, n = 6). Group 1 (negative control) received distilled water; Group 2, 3, and 4 were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the leaf extract, respectively; Group 5, 6 and 7 were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the root extract, respectively; and Group 8 was treated with the standard drug (Ranitidine, 50 mg/kg). Pylorus ligation was performed in all groups of fasted mice to induce gastric ulcer 1 h after the administration of treatments [29,30].

2.9.2. Repeated-graded dose study

Male mice were randomly assigned to eight groups (1–8, n = 6) and treated as mentioned in the single dose study. Treatments were administered daily at morning for 10 days. The animals were fasted for 24 h before administration of the 10th day treatment, and pylorus ligation was done 1 h after the administration of treatments [29,30].

2.10. Ethanol-induced ulcer model

2.10.1. Single-graded dose study

Forty eight fasted (for 24 h) male Swiss albino mice were randomly

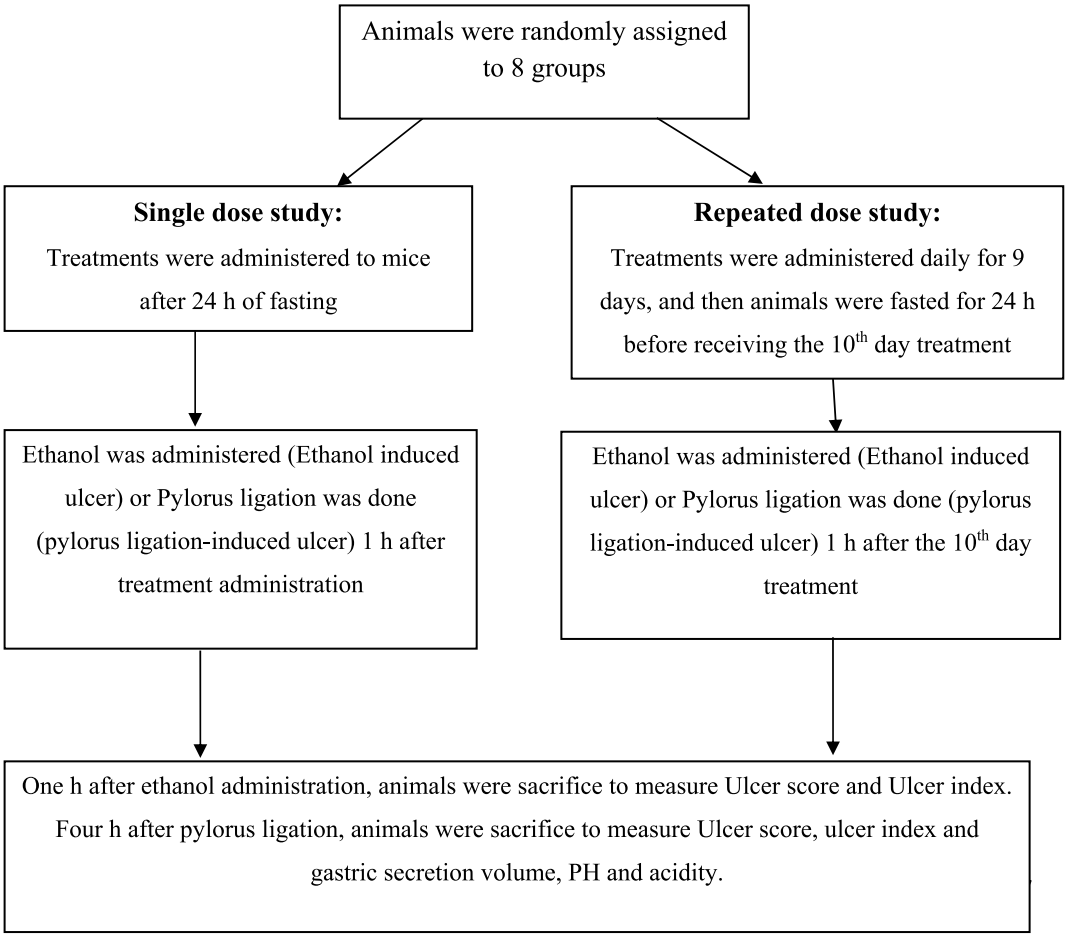

Fig. 1. Flow chart of the methods for screening the antiulcer activity of the study plant.

assigned to eight different groups (1–8, n = 6). Group 1 (negative control) received distilled water (10 ml/kg); Group 2, 3, and 4 were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the leaf extract, respectively; Group 5, 6 and 7 were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the root extract, respectively; and Group 8 was treated with the standard drug (Ranitidine, 50 mg/kg). Then, absolute ethanol (1 ml/200 g) was administered to each fasted mice to induce gastric ulcers 1 h after the administration of treatments [29,30].

The total mucosal area and total ulcerated area of the stomach were measured for each mouse using a graph paper. The ulcer index was then calculated using the following formula [33,34]

Ulcer index = 10/x, where x is the total mucosal area/ulcerated area.

Additionally, percentage inhibition of ulcer index was calculated as follows:

$$\%\ \text{Inhibition of Ulcer index} = \frac{\text{Ulcer index of Control} - \text{Ulcer index of Test}}{\text{Ulcer index of Control}} \times 100$$

2.10.2. Repeated-graded dose study

Male mice were randomly assigned to eight groups (1–8, n = 6) and treated as mentioned in the single dose study. Treatments were administered daily at morning for 10 days. The animals were fasted for 24 h before administration of the 10th day treatment, and 1 ml/200 g of absolute ethanol was administered to each fasted mice 1 h after the administration of treatments [29,30].

2.11. Evaluation of anti-ulcer activity

2.11.1. Pyloric ligation-induced ulcer model

Mice were fasted for 24 h before initiating the experiment, but they had free access to water. After 1 h of treatment administration, animals were anesthetized with the anesthetic agent, ether and the abdomen was opened by a small midline incision below the xiphoid process. Pylorus of the stomach was ligated after being slightly lifted out. The ligation was performed with caution to avoid traction to the pylorus or damage to its blood vessels. The stomach was placed back carefully to the normal position, and the abdominal wall was closed by interrupted sutures. Mice were sacrificed with overdose of pentobarbitone (150 mg/kg, i.p.) after 4 h of pyloric ligation. The abdomen was reopened, cardiac end of the stomach was dissected out, and the content was drained into a graduated centrifuge tube. The gastric content was then centrifuged at 2000 rpm for 10 min, and the supernatant volume and pH were recorded. The total acid content of gastric secretion was also determined by titration to pH 7.0 with 0.01 N NaOH. Each stomach was examined for lesions in the forestomach portion [30,31].

The 10-day period for the repeated dose study was selected based on previous studies [31,32], and the ulcer was induced on the 10th day of treatment after having the animals fasted for 24 h.

2.11.2. Macroscopic examination of the stomach

The stomach of each mouse was opened along the greater curvature and cleaned with normal saline to remove gastric contents and blood clots. The cleaned stomachs were then examined by a 10x magnifier lens for ulcer formation. The number of ulcers was counted for each mouse, and then each ulcer on the stomach was scored. Scoring of ulcer was done as follows [30]: Normal colored stomach (0), Hyperemia (0.5), Spot ulcer (1), Hemorrhagic streak (1.5), Deep ulcers (2), and Perforation (3).

The total ulcer score was recorded for each mouse. Additionally, percentage inhibition of ulcer score was calculated as follows:

$$\%\ \text{Inhibition of Ulcer score} = \frac{\text{Ulcer score of Control} - \text{Ulcer score of Test}}{\text{Ulcer score of Control}} \times 100$$

2.11.3. Determination of pH and total acidity

After measuring the volume of gastric secretion, pH of the centrifuged gastric secretion was measured using a digital pH meter. For the measurement of total acidity, an aliquot of 0.25 ml gastric secretion diluted with 0.25 ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator were added to it and titrated with 0.01 N NaOH until a permanent pink colour is observed. The volume of 0.01 N NaOH consumed was noted. The total acidity was expressed as mEq/L by the following formula [30]:

$$\text{Acidity} = (\text{Vol. of NaOH} \times \text{N} \times 100\ \text{mEq/L})/0.1$$

2.11.4. Ethanol-induced gastric ulcer

Mice were fasted for 24 h. Then, gastric ulcers were induced in the fasted mice by administrating ethanol (99%), 1 ml/200 g orally, after 60 min of hydromethanol extract and ranitidine treatment. They were kept in modified metabolic cages to prevent coprophagia during the experiment. The animals were sacrificed 1 h later with an overdose of pentobarbitone (150 mg/kg, i.p.), and the stomach was dissected and ulceration was scored as for the pyloric ligation-induced ulcer model [30].

The 10-day period for repeated-dose study is selected based on previous studies [31,32]. The ulcer was induced on the 10th day of treatment after having the animals fasted for 24 h. Scoring the ulcers was done as described above.

2.12. Data analysis

Data was expressed as mean ± standard error of the mean. Means of all parameters among groups were compared using one way ANOVA followed by Tuckey's multiple comparison test. P-value <0.05 was considered statistically significant. SPSS Version 20 Software was used for statistical analysis.

2.13. Ethical clearance

The animals were handled according to the international animal care and welfare [29], and the proposal of the study was submitted and

ethical clearance was obtained from the ethical review committee of college of medicine and health sciences, Wollo University (Reference number, WU/1137/05/11).

3. Result

3.1. Percentage yield of extraction with maceration

After maceration with hydromethanol, 35 g of dried dark-brown semisolid leaf extract (percentage yield, 28.45% w/w) and 33 g of dried yellowish brown semisolid root extract (percentage yield, 15% w/w) were harvested.

3.2. Acute oral toxicity study

The Acute toxicity study of both *Solanum incanum* leaf and root extracts didn't show any mortality and overt toxicity in the animals at the limit dose of 2 g/kg during the observation time. Thus, the median lethal dose (LD_{50}) of both leaf and root hydromethanol extracts of *Solanum incanum* is greater than 2 g/kg.

3.3. Preliminary qualitative phytochemical screening

Preliminary phytochemical analysis indicated the presence of flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, glycosides and steroids in the hydromethanol leaf and root extracts of *Solanum incanum* (Table 1).

3.4. Total phenolic and flavonoid content of the crude extracts

The total phenolic content and total flavonoid content of the hydromethanol leaf and root extracts of *Solanum incanum* is presented in the following table (Table 2).

3.5. Effects of the leaf and root extracts of Solanum incanum on pylorus ligation-induced ulcer

3.5.1. Single-graded dose study

Treatment with single-graded doses of leaf and root extracts of *S. incanum* didn't significantly ($P > 0.5$) reduce gastric secretion and total acidity compared to the negative control. Similarly both extracts didn't significantly ($P > 0.5$) enhance gastric pH compared to the negative control. Additionally, 100 mg/kg and 200 mg/kg of the leaf and root extracts failed to significantly reduce the ulcer score (US) and ulcer index (UI) compared to the negative control group. Both extracts significantly ($p < 0.05$) reduced US and UI at the dose of 400 mg/kg. Similarly, the standard drug (ranitidine, 50 mg/kg) reduced US ($P < 0.05$) and UI ($p < 0.001$) compared to the negative control. Additionally, ranitidine significantly enhanced ($p < 0.001$) gastric pH, and it significantly reduced the volume of gastric secretion ($p < 0.001$) and total acidity ($p < 0.01$) as compared to the negative control (Table 3).

Table 1
Preliminary qualitative Phytochemical screening of the hydromethanol leaf and root extract of *Solanum incanum*.

Phytochemicals	Result	
	Leaf	Root
Phenols	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	+	+
Alkaloids	+	+
Steroids	+	+
Glycosides	+	+
Anthraquinones	–	–

Key: +, present; –, absent.

Table 2
Total phenol content and total flavonoid content of the hydromethanol leaf and root extracts of *Solanum incanum*. (n = 3, X±SEM).

Extract	Total phenol content (mg Gallic Acid Equivalent per g)	Total flavonoid content (mg Quercetin Equivalent/g)
SILE	2.21 ± 0.29	0.23 ± 0.08
SIRE	2.17 ± 0.28	0.21 ± 0.02

SILE = *Solanum inanum* leaf extract, SIRE=*Solanum inanum* root extract.

Each value represents mean ± S.E.M, n = 6 for each treatment. [a]compared to DW; [b]compared to SILE100; [c]compared to SILE200; [d]compared to SILE400; [e]compared to SIRE100; [f]compared to SIRE200; [g]compared to SIRE400. [1]when $p < 0.05$, [2]when $p < 0.01$, [3]when $p < 0.001$. DW = distilled water, RAN = Ranitidine, SILE = *Solanum inanum* leaf extract, SIRE=*Solanum inanum* root extract, US=Ulcer Score, UI=Ulcer Index.

3.5.2. Repeated-graded dose study

Repeated daily dose administration of leaf and root extracts for 10 days didn't significantly ($P > 0.05$) affect volume of gastric volume, pH, and total acidity. Significant reduction in US was observed with 200 mg/kg ($p < 0.05$) and 400 mg/kg ($p < 0.01$) of the leaf extract after 10 days of repeated daily dose administration. In addition, 400 mg/kg of the leaf extract significantly ($p < 0.01$) reduced UI compared to the negative control group with a percentage reduction of 48.83%. Similarly, repeated daily administration of the root extract at the dose of 400 mg/kg significantly reduced ($p < 0.01$) US and UI compared to the negative control group with a percentage reduction of 48.37% and 48.67%, respectively. Ranitidine (50 mg/kg) significantly enhanced gastric pH ($p < 0.001$) and reduced volume of gastric secretion ($p < 0.05$), total acidity ($p < 0.01$), US ($p < 0.01$) and UI ($p < 0.01$) compared to the distilled water treated group after 10 days of administration (Table 4).

Each value represents mean ± S.E.M, n = 6 for each treatment. [a]compared to DW; [b]compared to SILE100; [c]compared to SILE200; [e]compared to SIRE100; [f]compared to SIRE200; [g]compared to SIRE400. [1]when $p < 0.05$, [2]when $p < 0.01$, [3]when $p < 0.001$. DW = distilled water, RAN = Ranitidine, SILE = *Solanum inanum* leaf extract, SIRE=*Solanum inanum* root extract, US=Ulcer Score, UI=Ulcer Index.

3.6. Effects of the leaf and root extracts of Solanum incanum on ethanol-induced ulcer

3.6.1. Single-graded dose study

Both extracts significantly reduced US at the dose of 200 mg/kg ($p < 0.05$) and 400 mg/kg ($p < 0.01$). Similarly, 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.001$) and 400 mg/kg ($p < 0.001$) of the leaf extract significantly reduced UI with a percentage reduction of 26.44%, 38.97% and 41.35%, respectively. Additionally, 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.001$) of the root extract showed significant reduction in UI compared to the negative control group with a percentage reduction of 29.42% and 38.37%, respectively. Ranitidine (50 mg/kg) significantly reduced ($p < 0.001$) US and UI compared to the negative control group (Table 5).

3.6.2. Repeated-graded dose study

Repeated daily dose administration of leaf and root extracts for 10 days showed a significant reduction in US and UI as compared to distilled water treated group. It was revealed that 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.01$) and 400 mg/kg ($p < 0.01$) of the leaf extract and 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.05$) and 400 mg/kg ($p < 0.01$) of the root extract caused a significant reduction in US. In addition, both the leaf and root extracts significantly reduced UI compared to the negative control group at the dose of 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.01$) and 400 mg/kg ($p < 0.01$). Similarly, ranitidine (50 mg/kg)

Table 3
Effects of single dose of *Solanum incanum* leaf and root extracts on pylorus ligation-induced ulcer.

Groups	Volume of gastric secretion	pH	Total acidity	US	% Inhibition of US	UI	% Inhibition of UI
DW	0.51 ± 0.01	4.37 ± 0.07	7.00 ± 1.03	6.17 ± 0.60	_	5.67 ± 0.57	_
RAN50	0.41 ± 0.01^{a3b3c2e3f3}	6.66 ± 0.07^{a3b3c3d3e3f3g3}	3.53 ± 0.41^{a2c2d2e2f2gl}	3.92 ± 0.30^{a1b1e2}	36.47%	3.07 ± 0.35^{a3b3c2e2f2}	45.86%
SILE100	0.50 ± 0.01	4.40 ± 0.09	4.23 ± 0.54^{e1}	6.08 ± 0.37	1.46%	5.82 ± 0.47	−2.65%
SILE200	0.49 ± 0.01	4.45 ± 0.13	7.07 ± 0.58	5.50 ± 0.32	10.86	5.08 ± 0.27	10.41
SILE400	0.46 ± 0.01	4.46 ± 0.41	6.95 ± 0.55	4.08 ± 0.37^{a1}	33.87%	3.98 ± 0.13^{a1}	29.81%
SIRE100	0.50 ± 0.02	4.40 ± 0.42	7.10 ± 0.58	6.42 ± 0.66	−4.05%	5.17 ± 0.33	8.82%
SIRE200	0.49 ± 0.01	4.41 ± 0.42	7.03 ± 0.58	5.58 ± 0.49	9.56%	5.07 ± 0.27	10.58%
SIRE400	0.46 ± 0.01	4.41 ± 0.15	6.57 ± 0.58	4.08 ± 0.24^{a1}	33.87%	4.00 ± 0.29^{a1}	29.45%

Table 4
Effects of repeated daily doses of *Solanum incanum* leaf and root extracts on pylorus ligation-induced ulcer.

Groups	Volume of gastric secretion	pH	Total acidity	US	% Inhibition of US	UI	% Inhibition of UI
DW	0.53 ± 0.02	4.20 ± 0.15	7.33 ± 0.92	6.45 ± 0.61	_	6.00 ± 0.65	_
RAN50	0.37 ± 0.02^{a1}	6.91 ± 0.23^{a3b3c3e3f3g2}	3.20 ± 0.47^{a2c1e1f1}	2.92 ± 0.42^{a2b1e1}	54.73%	2.73 ± 0.49^{a2b2}	54.5%
SILE100	0.45 ± 0.02	4.68 ± 0.28	4.73 ± 0.71	5.58 ± 0.58	13.49%	5.65 ± 0.61	5.83%
SILE200	0.46 ± 0.01	4.68 ± 0.29	6.73 ± 0.85	3.83 ± 0.56^{a1}	40.62%	3.95 ± 0.49	34.17%
SILE400	0.40 ± 0.04	5.46 ± 0.43	6.53 ± 0.89	3.33 ± 0.51^{a2}	48.37%	3.07 ± 0.55^{a2b1}	48.83%
SIRE100	0.44 ± 0.04	4.67 ± 0.49	6.77 ± 0.63	5.50 ± 0.62	14.73%	4.33 ± 0.48	27.83%
SIRE200	0.42 ± 0.05	4.74 ± 0.31	6.70 ± 0.67	4.67 ± 0.63	27.60%	4.17 ± 0.28	30.50%
SIRE400	0.41 ± 0.04	4.84 ± 0.35	6.15 ± 0.68	3.33 ± 0.36^{a2}	48.37%	3.08 ± 0.42^{a2b1}	48.67%

Table 5
Effects of single dose of *Solanum incanum* leaf and root extracts on ethanol-induced ulcer.

Groups	US	% Inhibition of US	UI	% Inhibition of UI
DW	5.17 ± 0.64	_	5.03 ± 0.58	_
RAN50	2.52 ± 0.18^{a3b1e1}	51.26%	2.47 ± 0.17^{a3e1}	50.89%
SILE100	4.00 ± 0.29	22.63%	3.70 ± 0.22^{a1}	26.44%
SILE200	3.50 ± 0.18^{a1}	32.30%	3.07 ± 0.29^{a3}	38.97%
SILE400	3.00 ± 0.29^{a2}	41.97%	2.95 ± 0.24^{a3}	41.35%
SIRE100	4.00 ± 0.29	22.63%	3.85 ± 0.16	23.46%
SIRE200	3.63 ± 0.19^{a1}	29.79%	3.55 ± 0.19^{a1}	29.42%
SIRE400	3.00 ± 0.29^{a2}	41.97%	3.10 ± 0.12^{a3}	38.37%

Each value represents mean ± S.E.M, n = 6 for each treatment. [a]compared to DW; [b]compared to SILE100; [e]compared to SIRE100. [1]when $p < 0.05$, [2]when $p < 0.01$, [3]when $p < 0.001$. DW = distilled water, RAN = Ranitidine, SILE = *Solanum inanum* leaf extract, SIRE=*Solanum inanum* root extract, US=Ulcer Score, UI=Ulcer Index.

Table 6
Effects of repeated daily doses of *Solanum incanum* leaf and root extracts on ethanol-induced ulcer.

Groups	US	% Inhibition of US	UI	% Inhibition of UI
DW	5.67 ± 0.21	_	5.08 ± 0.35	_
RAN50	1.58 ± 0.20^{a3b1e1f1}	72.13%	1.38 ± 0.17^{a3b1e1}	72.83%
SILE100	3.75 ± 0.69^{a1}	33.86%	3.28 ± 0.38^{a1}	35.43%
SILE200	3.08 ± 0.51^{a2}	45.68%	2.80 ± 0.62^{a2}	44.88%
SILE400	2.92 ± 0.24^{a2}	48.50%	2.42 ± 0.33^{a2}	52.36%
SIRE100	3.67 ± 0.40^{a1}	35.27%	3.25 ± 0.28^{a1}	36.02%
SIRE200	3.58 ± 0.42^{a1}	36.86%	2.93 ± 0.49^{a2}	42.32%
SIRE400	2.92 ± 0.35^{a2}	48.50%	2.42 ± 0.33^{a2}	52.36%

Each value represents mean ± S.E.M, n = 6 for each treatment. [a]compared to DW; [b]compared to SILE100; [e]compared to SIRE100; [f]compared to SIRE200. [1]when $p < 0.05$, [2]when $p < 0.01$, [3]when $p < 0.001$. DW = distilled water, RAN = Ranitidine, SILE = *Solanum inanum* leaf extract, SIRE=*Solanum inanum* root extract, US=Ulcer Score, UI=Ulcer Index.

significantly reduced ($p < 0.001$) US and UI compared to the distilled water treated group after 10 days of administration (Table 6 and Fig. 2).

4. Discussion

The current approved medications for the treatment of PUD (antacids, histamine H2-antagonists, proton pump inhibitors and anticholinergics) have several adverse reactions including gynecomastia, hematopoietic changes, thrombocytopenia, anaphylaxis reactions, acute interstitial nephritis, nephrotoxicity and hepatotoxicity [3,4,35–38]. These medications also produce significant drug-drug interactions that limit their potential use [5,6]. In developing countries like Ethiopia, treatment of PUD with conventional medications is very expensive. These limitations of currently used drugs for PUD call for development of new medications, and the search for novel molecules has been extended to medicinal plants that can offer products with better safety and efficacy.

In Ethiopia, *Solanum incanum* is traditionally used to treat PUD [13], and this study was conducted to evaluate the antiulcer activity of this medicinal plant using pylorus ligation-induced and ethanol-induced ulcers in mice.

Ligation of the pyloric end of the stomach causes accumulation of gastric acid and pepsin in the stomach which leads to mucosal digestion and formation of gastric ulcers. Pylorus ligation induced ulcer model is used to evaluate the anti-secretory action of drugs that reduce secretion of gastric aggressive factors such as acid and pepsin. The model is also useful for assessing the cytoprotective effects of drugs that increase secretion of mucus [30]. In the current study, *Solanum incanum* didn't significantly affect the volume, pH and total acidity of gastric secretion in pylorus ligation-induced ulcer model assuring the plant doesn't possess anti-secretory activity. This suggests cytoprotection as a possible mechanism for antiulcer activity of the plant.

Ethanol dissolves the protective gastric mucous membrane and expose the mucosa to the proteolytic and hydrolytic actions of HCl and pepsin. Moreover, ethanol stimulates gastric acid secretion and reduces blood flow to gastric wall. Additionally, Alcohol increases activity of

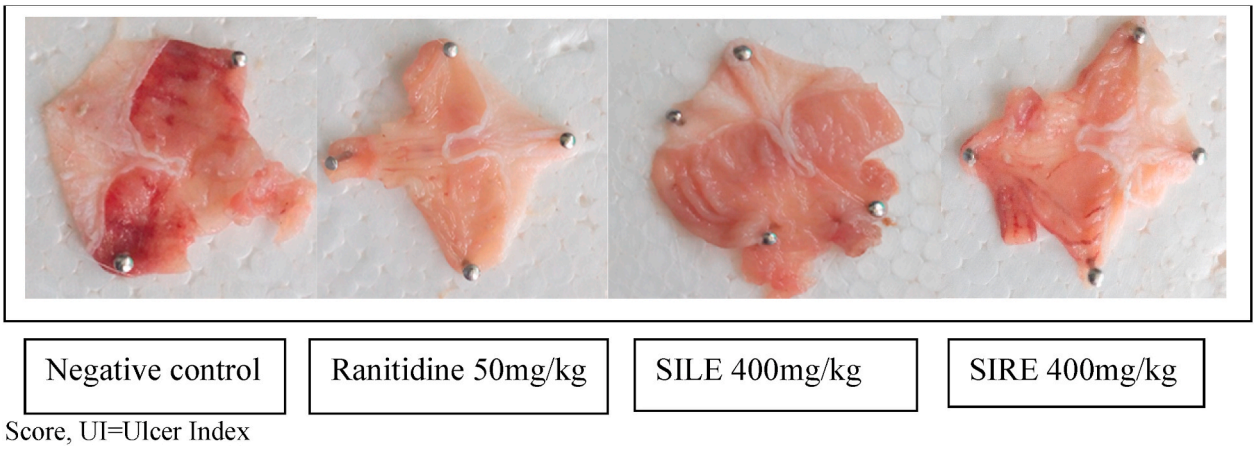

Fig. 2. Gross view of mice stomachs after repeated daily pre-treatment and ulcer induction with ethanol. SILE=*Solanum incanum* leaf extract, SIRE=*Solanum incanum* root extract.

xanthine oxidase. Ethanol also causes the release of superoxide anion and hydroperoxy free radicals, and hence increased oxidative stress in the tissues causing lipid peroxidation. The ethanol-induced ulcer model is useful for studying the efficacy of potential drugs that have cytoprotective and/or antioxidant activities [30].

In this study the leaf and root extracts of *Solanum incanum* reduce ulcer score (measure of depth of ulcer) and ulcer index (measure of the ulcer area) in both pylorus ligation-induced and ethanol-induced ulcers. Previous studies indicated that the antiulcer activity of medicinal plants is mainly due to the presence of phenolic compounds, flavonoids and saponins [15–17]. In the current study, Preliminary phytochemical study of the hydromethanol leaf and root extracts of *Solanum incanum* indicated the presence of phenols, flavonoids and saponins. Thus, the antiulcer activity of this medicinal plant might be secondary to the presence of these phytochemicals known to have antiulcer activity.

Oxidative stress and inflammation are among the principal pathogenesis mechanisms of PUD [21,22]. Previous studies have reported *Solanum incanum* has antioxidant [18,19] and anti-inflammatory [39] activities, and these pharmacologic activities may be involved in the antiulcerogenic effect of the plant.

It has been well documented that natural products played critical roles in modern drug development, especially for antibacterial and antitumor agents. Despite the current preoccupation with synthetic chemistry as a vehicle to discover and manufacture drugs, the contribution of plants to disease treatment and prevention is still enormous [40]. Thus, *S. incanum* can be used as a potential source of new antiulcer drug.

As a limitation, this study didn't isolate and identify the active compounds involved in the antiulcer activity of the plant. Additionally, the exact molecular mechanism for antiulcer activity was not determined.

5. Conclusion

This study showed that hydromethanol leaf and root extracts of *Solanum incanum* have significantly reduced ulcer score and ulcer index revealing the antiulcerogenic activity of the plant. The extracts didn't affect acid secretion suggesting cytoprotection as a possible mechanism of antiulcer activity.

Declarations

Ethics approval and consent to participate

The experiment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals [41], and the study has been approved by the ethical review committee of college of medicine and health sciences, Wollo University (Reference number, WU/1137/05/11).

Consent to publish

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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