



## A COMPARATIVE STUDY ON ANTIULCER ROLE OF *TINOSPORA CORDIFOLIA* AND *SPINUS WALLICHIANA* IN COLD RESTRAINT STRESS MODEL

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### Abstract

Peptic ulcer is acid-induced injury of intestinal system that is generally situated in stomach or upper segment of duodenum. The present research was based on the antiulcer role of *Tinospora cordifolia* and *Pinus wallichiana* in cold restraint stress model. Leaves of *Tinospora cordifolia* and *Pinus wallichiana* were obtained from the Katihar region and Uttarakhand region, India. These were identified and authenticated by a botanist. Both the leaves were washed making dust-free and dried at room temperature or shade. Animal House, School of Pharmacy, Al-Karim University provided the Wistar rats of either sex weighing 130–160g. The animals were kept in good health, with room temperatures of 25°C and a 12-hour light/dark cycle. All the rats were divided into 6 groups (n=6) i.e., group 1 administered normal saline, group 2 administered Ranitidine (20mg/kg), group 3 administered *T. cordifolia* ethanolic leaves extract (TCELE) (200mg/kg, p. o.), group 4 administered *T. cordifolia* ethanolic leaves extract (TCELE) (400mg/kg, p. o.), group 5 administered *Pinus wallichiana* ethanolic leaves extract (PWELE) (200mg/kg, p. o.) and group 6 administered *Pinus wallichiana* ethanolic leaves extract (PWELE) (400mg/kg, p. o.) up to 15 days. The Cold Restraint Stress Model was carried out for ulcer induction. Various parameters were estimated including ulcer index, percentage inhibition, pH, volume of gastric acid, free acidity and total acidity. It resulted, in all the parameters evaluated, that ethanolic leaves extract of *Tinospora cordifolia* and *Pinus wallichiana* showed a significant anti-ulcerogenic activity when compared to reference drug- Ranitidine. However, between these two herbal extracts, *Pinus wallichiana* was found more potent and effective as compared to *Tinospora cordifolia*. The response was observed as dose dependent. In conclusion, these have been approved for having anti-ulcer potential in preclinical studied. Clinical trials are needed to confirm its actual property by which they can be used in humans as well. Its action might be due to presence of flavonoids and related active moieties.

**Keywords:** *Tinospora cordifolia*, *Pinus wallichiana*, anti-ulcer activity, cold restraint stress model, ulcer index.

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## INTRODUCTION

Peptic ulcer is acid-induced injury of intestinal system that is generally situated in stomach or upper segment of duodenum. According to Zhang et al. (2014), it is distinguished by bared mucosa with imperfections extending into the sub-mucosa. Intestinal ulcer is four times as common than gastric ulcer. In actuality, duodenal ulcers affect men more frequently than they do women. In the early stages of mucosal injury, individual susceptibility is essential since only a small proportion of people with *H. pylori* infection or who use NSAIDs experience peptic ulcers. According to Datta and Roychoudhury (2015), functional polymorphisms in a number of cytokine genes are associated with peptic ulcers. Epigastric pain, bloating, abdominal load, nausea, emesis, frequent, recurrent weight loss, dysphagia that worsens over time, excessive GI bleeding, iron deficiency anaemia, and cancer in ancestors are some of the typical and concerning symptoms of PUD.

The most often afflicted organs are the stomach and upper duodenum. The distal duodenum, jejunum, and lower oesophagus are all affected. While discomfort in individuals with a duodenal ulcer typically appears 2-3 hours after a meal, patients with a stomach ulcer typically experience epigastric pain 15-30 minutes after a meal (Malik et al. 2021). Aspirin users have a twice as high chance of developing peptic ulcer complications as compared to NSAID users, who had a four times higher risk. Upper gastrointestinal haemorrhage is more likely when NSAIDs or aspirin are combined with anticoagulants, corticosteroids, or SSRIs (Kuna et al. 2019).

### Plant profile

***Tinospora cordifolia*:** Numerous ancient books recommend the use of *Tinospora cordifolia* (Guduchi), an important drug in the Ayurvedic system of medicine, for the treatment of various ailments. Many different illnesses include jaundice, fever, and skin diabetes and illnesses (Sinha et al. 2004) The *T. cordifolia* is a substantial, green herbaceous, glabrous, and deciduous plant. *T. cordifolia* is spread across China and India. In Hindi, the name is Guduchi. It is a mythological phrase from Hinduism that refers to *T. cordifolia* (Bharathi et al. 2018). It is used to describe the celestial elixir that is kept the body in old age and works to rejuvenate it. *T. cordifolia* is a typical climbing shrub that grows on the south Indian peninsular plains' tropical deciduous forest.

### Taxonomy

Kingdom: Plantae  
Order: Ranunculales  
Family: Menispermaceae  
Genus: *Tinospora*  
Species: *T. cordifolia*

According to reports, the plant contains berberine, columbin, tinosporin, and tinosporin acid. In the conventional medical system, it has a solid reputation for treating a wide range of illnesses. *T. cordifolia* is a typical greenish-yellow flowering herbaceous vine shrub (Upadhyay et al. 2010). Various actions of this plant, including anti-periodic, immunomodulatory, anti-diabetic, anti-leprotic, anti-inflammatory, antispasmodic, antioxidant, anti-arthritic, anti-allergic, antimalarial, anti-stress, and anti-neoplastic activity, have recently been identified (Jeyachandran et al. 2003).

***Pinus wallichiana*:** The *Pinus* species that are native to the Hindu Kush Mountains, Karakoram Mountains, and Himalayan Ranges. With an altitude range of 1800 metres to 4300 metres, this plant can be found in extensive and luxuriant growth all along the Himalayan ranges, starting in eastern Afghanistan and continuing through Pakistan, India, Nepal, Bhutan, Myanmar, and China (Ghimire et al. 2010). The plant loves to thrive in colder climates and can be found in areas with high altitudes and little rainfall or low altitudes with a lot of rainfall, either in the form of pure or mixed forests (Rahman et al. 2017).

### Taxonomy

Kingdom: Plantae  
Order: Pinales  
Family: Pinaceae  
Genus: *Pinus*  
Species: *wallichiana*

With a straight trunk and small, downcurved limbs that are longer in solitary trees, *Pinus wallichiana* grows to a height of more than 50 metres, giving the tree a dome-like appearance. When young, the bark of the trunk is smooth and resinous. As it ages, the bark becomes corky and grey with shallow fissures. Five leaves or needles per fascicle, the leaves or needles range in length from 10 to 20 cm and are blue green in colour. At maturity, female cones are very resinous and pale brown. 20 cm to 30 cm long female cones are found in groups of one to six. The cone is upright when young, turning pendent as it matures, and it is light brown in colour. The colour of the apophysis is light brown.

The pollen sacs on the lower surface of each of the male cones' ovoid or globose, short-lived scales or microsporophylls (Sharma et al.2018).

## MATERIALS AND METHODS

### Experimental requirements

Leaves of *Tinospora cordifolia* and *Pinus wallichiana*, Ranitidine, Water bath, distilled water, albino rats of either sex, rotatory evaporator, weighing machine, ethanol.

### Collection, Identification & Authentication of plant

Leaves of *Tinospora cordifolia* and *Pinus wallichiana* were obtained from the Katihar region, Bihar, India. These were identified and authenticated by a botanist. Both the leaves were washed making dust-free and dried at room temperature or shade. The dried leaves are rendered into coarse powders and then finally into fine ones. The powder is weighed and soaked into ethanol for 15 days with gradual stirrings, in two different beakers. A rotating evaporator is used to dry the brownish, semisolid extract obtained under partial vacuum. The yield of the leaf extract is calculated as a percentage (Khan et al. 2020).

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

### Preparation of animals

Animal House, School of Pharmacy, Al-Karim University provided the Wistar rats of either sex weighing 130–160g. The animals were kept in good health, with room temperatures of 25°C and a 12-hour light/dark cycle. The relative humidity is kept at 44-56%, and the rats were provided a regular rodent diet and free access to water. The animals were kept on fasting but have free access to water until 1 hour before the ulcers are induced (Bhajoni et al. 2016).

### Experimental protocols

All the rats were divided into 6 groups (n=6) as followings-

Group 1 was administered normal saline once a day for 15 days.

Group 2 was administered Ranitidine (20mg/kg) orally, for 15 days.

Group 3 was administered *T. cordifolia* ethanolic leaves extract (TCELE) (200mg/kg) orally, for 15 days.

Group 4 was administered *T. cordifolia* ethanolic leaves extract (TCELE) (400mg/kg) orally up to 15 days.

Group 5 was administered *Pinus wallichiana* ethanolic leaves extract (PWELE) (200mg/kg) orally, for 15 days.

Group 6 was administered *Pinus wallichiana* ethanolic leaves extract (PWELE) (400mg/kg) orally up to 15 days.

### Cold Restraint Stress Model- Ulcer induction

Rats from each group were put into a tub that measured 90cm×50cm×70cm (L×W×H) and had a water level of 30cm. For 15 days straight, stress was applied once every day. After stress was applied to the rats on day 14, they were promptly dissected under anesthetic ether, and blood was taken in microtubes. To get serum, blood was centrifuged at 1610×g for 20 min at 4°C. The serum was then collected and kept at -20°C until testing.

### Evaluation parameters

#### 1. Ulcer index and percentage inhibition

By pinching the lower length of the oesophagus, the pyloric segment of the stomach is dissected out. The ulcer index is measured in the glandular part of the stomach (UI). The ulcer area is calculated by adding the width and length of each lesion, as well as the total area of every incision (mm<sup>2</sup>) (Abdulla et al. 2009).

The PI is calculated by the below mentioned formula-

$$\text{PI} = [(\text{UA}_{\text{control}} - \text{UA}_{\text{treated}}) \div \text{UA}_{\text{control}}] \times 100$$

The gastric content is taken into tubes and after centrifugation it is used to test for different parameters-

- pH
- Volume of gastric content
- It is (content of gastric- lavage) titrated against 0.01N NaOH to determine free & total acidity (Dinda, 2012).

#### 2. pH detection

Gastric content is taken out and kept in contaminated free petri-dish. After, the pH is easily measured by using digital pH meter. It confirms about the acidity level in the rodent.

#### 3. Gastric Volume determination

In this test, gastric content of stomach of rat is taken out and filled into measuring cylinder to confirm the actual volume of gastric fluid. It confirms about the level of acidity developed in the rat and effect of the drug.

#### 4. Free & total acidity

In this procedure, firstly gastric content is taken out separately. It is (content of gastric- lavage) titrated against 0.01N NaOH to determine free & total

acidity. It confirms the level of acidity and beneficial effect of drug given.

## RESULTS AND DISCUSSION

### 1. Ulcer index and percentage inhibition

Group 1 exhibited UI score of  $1.79 \pm 0.03$  but PI was observed Nil. Whereas, group 2 (Ranitidine 20mg/kg) demonstrated UI as  $0.31 \pm 0.02$  and PI as 93.04 that is highly significant as it serves at standard drug. So, UI was achieved minimum after ranitidine administration and PI was noted maximum in terms of ulceration inhibition. Group 3 reduced the ulcer index by up to  $0.57 \pm 0.03$  and PI

was observed in ascending manner as 67.29. Whereas, group 4 was treated with TCELE (400mg/kg, *p. o.*) and demonstrated the ulcer index as  $0.48 \pm 0.04$  and percentage inhibition was recorded as 85.03 which is near to standard drug treatment.

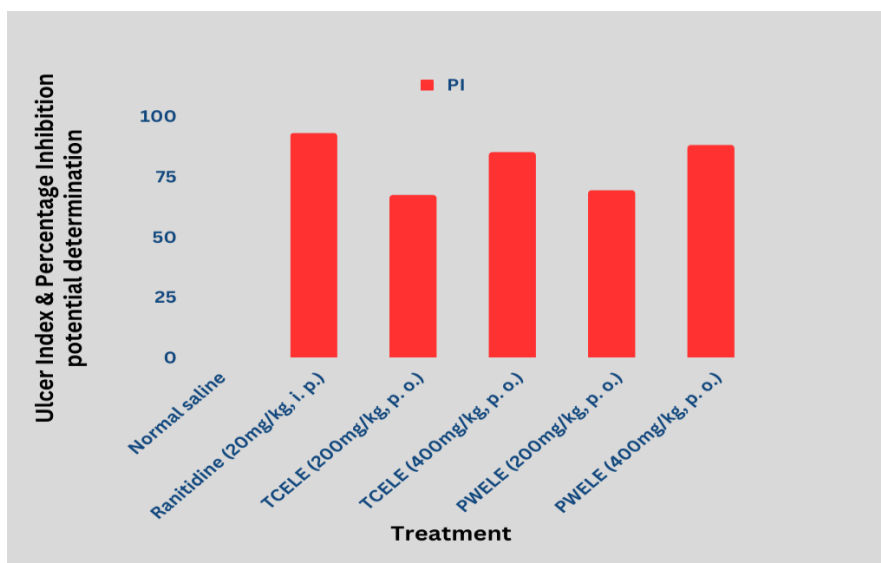
So, in this model the result indicates that PWELE (400mg/kg, *p. o.*) in higher dose is much effective which is comparable to standard group.

The following table confers the UI and PI effect of *Tinospora cordifolia* and *Pinus wallichiana*-

**Table 1. Ulcer Index & Percentage Inhibition determination**

Group	Treatment	UI	PI
1	Normal saline	$1.79 \pm 0.03$	Nil
2	Ranitidine (20mg/kg, <i>i. p.</i> )	$0.31 \pm 0.02$	93.04
3	TCELE (200mg/kg, <i>p. o.</i> )	$0.57 \pm 0.03$	67.29
4	TCELE (400mg/kg, <i>p. o.</i> )	$0.48 \pm 0.04$	85.03
5	PWELE (200mg/kg, <i>p. o.</i> )	$0.55 \pm 0.06$	69.24
6	PWELE (400mg/kg, <i>p. o.</i> )	$0.43 \pm 0.02$	88.05

Significance level denoted by \* significant at  $P < 0.05$ , N=6, values are in Mean  $\pm$  SEM



**Fig 1. Graphical presentation of percentage inhibition**

### 2. pH detection

Group 1 which administered normal saline and pH was observed as  $2.28 \pm 0.19$  after 15 days of continuous exposure of dosing frequency once per day. Group 2 given Ranitidine in the dose of 20mg/kg showed optimum and increased pH in the range of  $4.46 \pm 0.16$ . Group 3 administered *Tinospora cordifolia* (200mg/kg, *p. o.*) and pH was recorded as  $2.83 \pm 0.11$  whereas Group 4 given

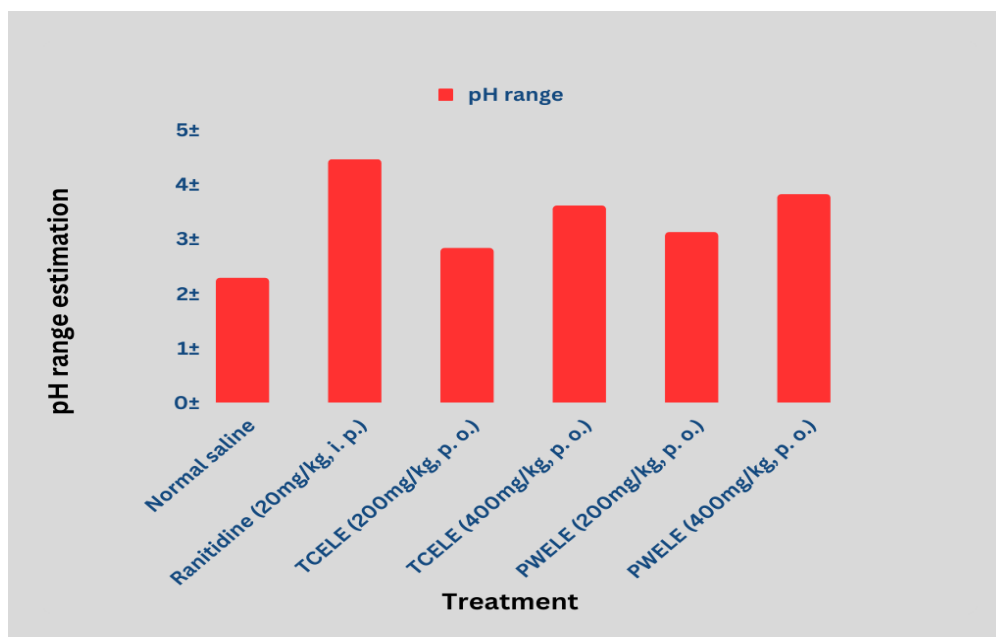
*Tinospora cordifolia* (400mg/kg) for 15 days and showed pH as  $3.61 \pm 0.13$ . While Group 5 and Group 6 were treated with *Pinus wallichiana* at the dose of 200mg/kg and 400mg/kg, respectively that exhibited pH of  $3.12 \pm 0.16$  and  $3.82 \pm 0.12$ , respectively.

The following table depicts the response of herbal extracts on pH-

**Table 2. pH range estimation**

Group	Treatment	pH range
1	Normal saline	2.28±0.19
2	Ranitidine (20mg/kg, i. p.)	4.46±0.16
3	TCELE (200mg/kg, p. o.)	2.83±0.11
4	TCELE (400mg/kg, p. o.)	3.61±0.13
5	PWELE (200mg/kg, p. o.)	3.12±0.16
6	PWELE (400mg/kg, p. o.)	3.82±0.12

Significance level denoted by \* significant at P<0.05, N=6, values are in Mean± SEM



**Fig 2. Graphical presentation of pH range estimation**

### 3. Volume of gastric content

Group 2 administered with Ranitidine in the dose of 20mg/kg recorded for volume of gastric content as 5.32±0.17ml. The volume of gastric content (ml) for Group 3 treated with *Tinospora cordifolia* was observed as 8.63±0.26ml whereas group 4 exhibited and decreased level of secreted volume of

gastric content as 7.24±0.27ml which is much significant and comparable to standard group.

The *Pinus wallichiana* treated rats at dose of 400mg/kg showed gastric content as 6.64±0.20ml. The following table demonstrates the decreased volume of gastric content (ml)-

**Table 3. Volume of gastric content estimation**

Group	Treatment	Volume of gastric content (ml)
1	Normal saline	12.29±0.47
2	Ranitidine (20mg/kg, i. p.)	5.32±0.17
3	TCELE (200mg/kg, p. o.)	8.63±0.26
4	TCELE (400mg/kg, p. o.)	7.24±0.27
5	PWELE (200mg/kg, p. o.)	7.87±0.16
6	PWELE (400mg/kg, p. o.)	6.64±0.20

Significance level denoted by \* significant at P<0.05, N=6, values are in Mean± SEM

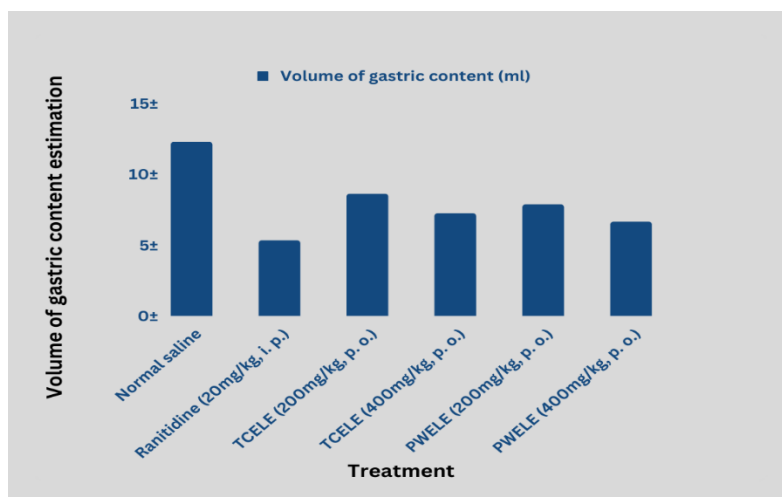


Fig 3. Graphical presentation of Volume of gastric content estimation

#### 4. Free acidity

Group 1 administered with distilled water showed free acidity with value of  $38.29 \pm 1.40$  mEq/l which is ineffective completely. Group 2 treated with ranitidine in the dose of 20mg/kg, all the animals exhibited mean value of free acidity as  $21.26 \pm 2.37$  mEq/l. Animals treated with test drug-

*Tinospora cordifolia* and *Pinus wallichiana* produced free acidity in the range of  $27.11 \pm 1.22$  mEq/l and  $25.53 \pm 1.13$  mEq/l at the dose of 400mg/kg. While, the effect was more dominant in *Pinus wallichiana* than *Tinospora cordifolia*. The following table depicts the free acidity-

Table 4. Free acidity (mEq/l) determination

Group	Treatment	Free acidity (mEq/l)
1	Normal saline	$38.29 \pm 1.40$
2	Ranitidine (20mg/kg, i. p.)	$21.26 \pm 2.37$
3	TCELE (200mg/kg, p. o.)	$29.12 \pm 1.30$
4	TCELE (400mg/kg, p. o.)	$27.11 \pm 1.22$
5	PWELE (200mg/kg, p. o.)	$28.62 \pm 1.24$
6	PWELE (400mg/kg, p. o.)	$25.53 \pm 1.13$

Significance level denoted by \* significant at  $P < 0.05$ , N=6, values are in Mean  $\pm$  SEM

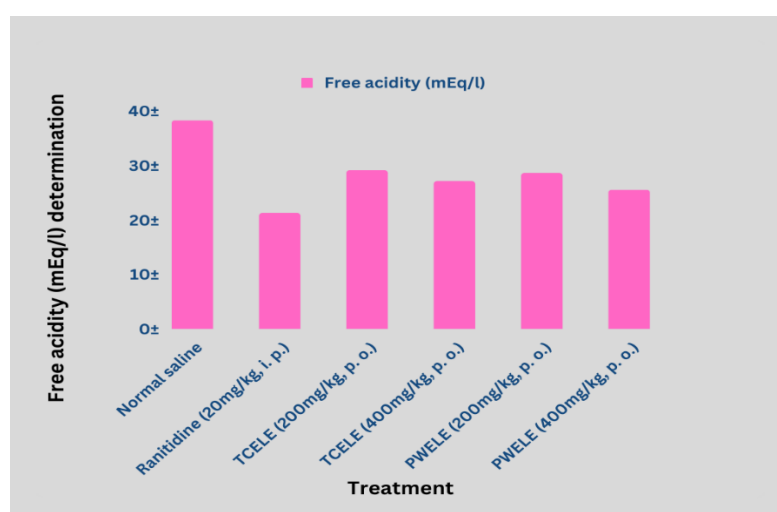


Fig 4. Graphical presentation of free acidity (mEq/l) determination

#### 5. Total acidity

Total acidity was noted as  $74.19 \pm 1.85$  mEq/l in group 1 which was treated with normal saline. Group 2 treated with Ranitidine in the dose of 20mg/kg, all the animals exhibited mean value of total acidity as  $53.37 \pm 2.30$  mEq/l. Animals treated with test drug-

20mg/kg exhibited total acidity as  $53.37 \pm 2.30$  mEq/l. Whereas, group 4 and group 6 showed total acidity as  $63.34 \pm 1.27$  mEq/l and  $58.11 \pm 1.29$  mEq/l that were administered

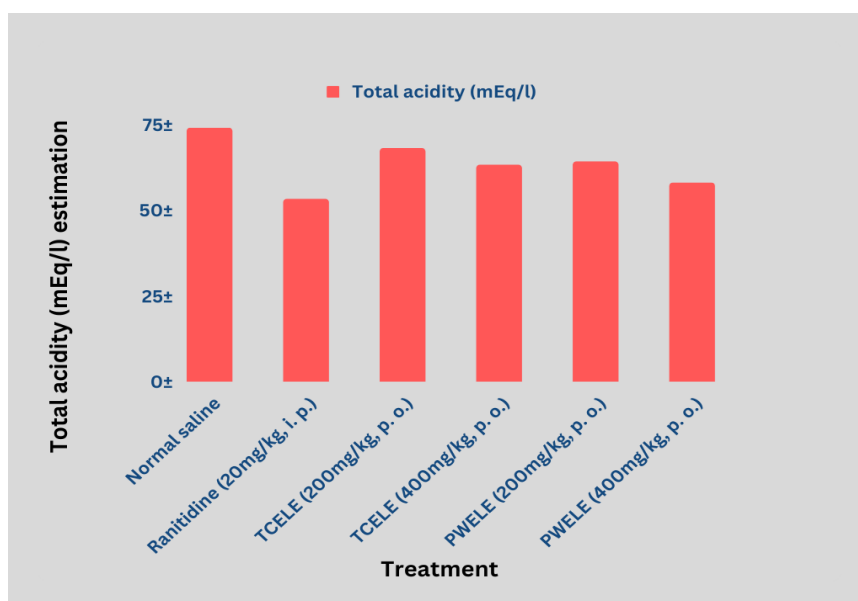
*Tinospora cordifolia* and *Pinus wallichiana* leaves extract at 400mg/kg for persistently 15 days.

The following table shows the response of herbal extracts on total acidity-

**Table 5. Total acidity (mEq/l) estimation**

Group	Treatment	Total acidity (mEq/l)
1	Normal saline	74.19±1.85
2	Ranitidine (20mg/kg, i. p.)	53.37±2.30
3	TCELE (200mg/kg, p. o.)	68.20±1.26
4	TCELE (400mg/kg, p. o.)	63.34±1.27
5	PWELE (200mg/kg, p. o.)	64.31±1.46
6	PWELE (400mg/kg, p. o.)	58.11±1.29

Significance level denoted by \* significant at P<0.05, N=6, values are in Mean± SEM



**Fig 5. Graphical presentation of total acidity (mEq/l) estimation**

It resulted, in all the parameters evaluated, that ethanolic leaves extract of *Tinospora cordifolia* and *Pinus wallichiana* showed a significant anti-ulcerogenic activity when compared to reference drug- Ranitidine. However, between these two herbal extracts, *Pinus wallichiana* was found more potent and effective as compared to *Tinospora cordifolia*. The response was observed as dose dependent.

### CONCLUSION

*Tinospora cordifolia* as well as *Pinus wallichiana* are high in nutrients and bioactive chemicals, and they contain a wide range of pharmacological qualities that may aid in the prevention and treatment of chronic disorders. *Pinus wallichiana* leaves extract has higher levels of anti-ulcerogenic potential.

In conclusion, these have been approved for having anti-ulcer potential in preclinical studied. Clinical trials are needed to confirm its actual property by

which they can be used in humans as well. Its action might be due to presence of flavonoids and related active moieties.

### CONFLICT OF INTEREST

None

### SOURCE OF FUNDING

Nil

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