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# Evaluation of anti-ulcer activity of ethanolic extracts of indigofera tinctoria on albino rats

*Dhanapal Venkatachalam*
*vddpaul@gmail.com*
*Sanjo College of Pharmaceutical Studies, Vellapara, Kerala*

*Samuel Thavamani*
*samtmani78@rediffmail.com*
*Sanjo College of Pharmaceutical Studies, Vellapara, Kerala*

*Muddukrishnaiah*
*krishna123muddu@gmail.com*
*Sanjo College of Pharmaceutical Studies, Vellapara, Kerala*

*Sukanya Vijayan*
*sukanyavjn@gmail.com*
*Sanjo College of Pharmaceutical Studies, Vellapara, Kerala*

*Vinod K R*
*sanjocps@gmail.com*
*Sanjo College of Pharmaceutical Studies, Vellapara, Kerala*

## ABSTRACT

***Ulcer is the most common prevalent gastro intestinal disorder, which affects approximately 10 -15% of people in the world. It makes major global health problem today. Ulcer is an open sore, it can be developed inside the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer). Both the ulcers are also commonly referred to as peptic ulcers. Objectives: The present study was carried out to investigate the anti-ulcer activity of Ethanol extract of Indigofera tinctoria on albino rats. Materials and Methods: The present study was carried out by pylorus ligation induced ulcer models in albino rats. The antiulcer activity of ethanol extracts of Indigofera tinctoria (125, 250 mg/kg p.o. 7 days) was compared with standard drugs (Famotidine). In pyloric ligation induced ulcer model, the studied parameters were gastric volume, pH, total acidity, ulcer score, and ulcer index. Results: In pyloric ligation model; the volume of gastric content, total acidity, ulcer score were significantly decreased at p<0.01 and pH of the gastric juice were significantly increased at p<0.05 in EEIT treated groups as compared to control group. All the doses of EEIT showed the dose-dependent antiulcer effect as well as significant (p<0.01) reduction in the ulcer index as compared to control group in all the experimental models. The ethanol extract of EEIT at 250 mg/kg has more potent antiulcer activity than 125 mg/kg of ethanol extract of EEIT. Conclusion: The results of the study indicate that the EEIT has better potential against ulcer which supports the traditional claims in folklore medicine.***

***Keywords***: *Antiulcer Effect, Indigofera Tinctoria, Ethanol, Pylorus Ligation, Ulcer Index*

## 1. INTRODUCTION

Ulcer is an open sore that develops on the inside lining of the stomach (a gastric ulcer) or the small intestine (a duodenal ulcer). Both types of ulcers are also referred to as peptic ulcers. The most common symptom of a peptic ulcer is a burning or gnawing pain in the center of the abdomen (stomach). In the past, it was mistakenly thought that the main causes of peptic ulcers were lifestyle factors, such as diet, smoking, alcohol, and stress. While these factors may play a limited role, it is now known that the leading cause of peptic ulcers is a type of bacteria called H. pylori. It can infect the stomach and small intestine; and in some people, the bacteria can irritate the inner layer of the stomach and small intestine, leading to the formation of an ulcer [1] Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors.[2] Painkillers known as non-steroidal anti-inflammatory drugs (NSAIDs), which include aspirin and ibuprofen, are the second most common cause of peptic ulcers. These types of painkillers can irritate the lining of the stomach and small intestine in some people, particularly if they are taken on a long-term basis. A number of drugs, including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents, are available for the treatment of peptic ulcer, but most of these drugs produce several adverse reactions, including toxicities, and may even alter biochemical mechanisms of the body upon chronic usage.[3] The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer 4. In this modern era also, 75-80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility

with human body [5] *Indigofera tinctoria* is a leguminous plant which is widespread across tropical regions around the globe, as it had been cultivated and highly valued for centuries as the main source of indigo dye, leading to its common names 'true indigo' and 'common indigo' before commercial synthetic indigo production came into use in 1897 and reduced the world's total plant-derived indigo production to 4% by 1914 (Lemmens and Wulijarni-Soetjipto, 1991; PROTA, 2014). Medicinal uses include the juice of the leaves as a prophylactic against hydrophobia, and as a decoction for blennorrhagia; plant extract as treatment for epilepsy, nervous disorders, bronchitis, and as an ointment for sores, old ulcers, and haemorrhoids; and roots for hepatitis, scorpion bites, and urinary complaints (Duke, 1981; Lemmens and Wulijarni-Soetjipto, 1991). Hence the present work was undertaken to investigate the antiulcer activity of ethanol extract of *Indigofera tinctoria* in albino rats.

## 2. MATERIALS AND METHODS

### Plant material

*Indigofera tinctoria* leaf was collected from in and around palakkad, Kerala, India and authenticated by Dr.P.Jayraman, Director of Plant Anatomy Research Centre Chennai The *Indigofera tinctoria* leaf was identified and deposited at Department of Pharmacognosy, Sanjo college of pharmaceutical studies, Vellapara, Palakkad with the voucher number SCPDPCOG/IT/2018. The fresh leaf was separated and kept for shade drying. Dried leaf material was powdered using a mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an airtight container.

### Extraction of Plant material

The leaves of *I.tinctoria* were washed thoroughly in water to remove foreign matter and allowed to shade dry with a relative humidity of 40–45%. Then, the leaves were powdered in roller grinder and passed through a sieve (No. 40). Then, the fine powder (Approx. 150 gm) was defatted with petroleum ether and extracted with 1 liter of 95% ethanol at room temperature by using Soxhlet apparatus for 72 hours. The resultant extract was filtered and concentrated in a rotary evaporator under reduced pressure to obtain a thick semi-solid which was stored at −20°C until required. The yield of the extract was found to be 7.2 %w/w.

### Chemicals and Drugs

Famotidine and Anaesthetic ether were purchased from Sigma Co. (Sigma St. Louis, MO). Absolute ethanol was of analytical grade and was purchased from Merck (German). The other reagents were of analytical grade.

### Animals

Swiss albino mice 90-170 gms maintained in the Animal house facility of the Department of Pharmacology, Sanjo College of pharmaceutical studies, were used in these experiments. The animals were maintained on standard small animal feeds (Excel feed, Ilorin) and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) under the reference no. 1332/DPCG//18/CPCSEA and CPCSEA guidelines adhered to during the maintenance and experiment. This research was carried out in accordance with the rules governing the use of laboratory animals as accepted internationally. The experiment was conducted between the hours of 900 h and 1600 h. The experimental groups consisted of six animals. They were maintained at constant room temperature (22° ± 1 °C) and submitted to12 h light/dark cycle with free access to food and water.

## 3. EXPERIMENTAL PROCEDURE

### Acute oral toxicity study

Acute oral toxicity was conducted as per OECD guidelines (Organisation of Economic Cooperation and Development) 423 (Acute toxic class method). The acute toxic class method is a stepwise procedure of three animal of a single-sex per step. Depending on the mortality and/or moribund status of animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for the acceptable data-based scientific conclusion. The method uses defined doses, (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemicals which causes acute toxicity. The method previously described by Lorke was adopted [6]

### Animal group and treatment schedule for Anti-ulcer activity:

In all the experimental models, male *albino* rats were selected and divided into four groups of six animals each. Animals were fasted for 24 hours before the study but had free access to water. Group I treated as vehicle control, received only distilled water; group II, III treated as treatment groups, received the graded dose of ethanol extract of *I.tinctoria* at 125, 250 mg/kg, (P.O.) for 7 days (once in a day) respectively and group IV as standard group, received Famotidine 30 mg/kg (P.O.).

### Procedure for Pylorus ligation induced ulcer model: [7-8]

Anaesthetized the overnight fasted rat with anesthetic ether. Secure the rat on the operating table. Give an incision of 1 cm long in the abdomen just below the sternum. Expose the stomach. Pass a thread around the pyloric sphincter and apply a tight knot. Close the abdomen wall by putting the sutures. Clean the skin from any blood spots and bleeding. Apply collodion over the wound. Keep the rat in a separate cage and allow it to recover. To another rat inject (Famotidine 30mg/kg) after 15 mts perform pyloric ligation as described above step. After 4 hr of pyloric ligation sacrifices both the animals by decapitation. Open the abdomen and the Oesophageal and (cardiac end) of the stomach. Cut and remove the entire stomach from the body of the animal. Give a small cut to the pyloric region just above the knot and collect the contents of the stomach in a graduated centrifuge tube. Open the stomach along the greater curvature and wash it slowly under the running tap water. Put it on the glass slide and observe under 10X magnifications for ulcers. Score the ulcers as below:

0-Normal colored stomach
0.5- Red coloration
1- Ulcers spot

1.5- Haemorrhagic streaks
2- Ulcers ≥ 3 but ≤ 5
3- Ulcers > 5

Mean ulcer score for each animal is expressed as ulcer index[9].
Ulcer index ($U_I$) was measured by using following formula:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where $U_I$ (Ulcer Index);
$U_N$ (Average number of ulcers per animal);
$U_S$ (Average number of severity score);
$U_P$ (Percentage of animals with ulcers)

Centrifuge the gastric content at1000 rpm for 10 mts Note the volume. Pipette out 1 ml of supernatant liquid and dilute it to 10 ml with distilled water. Note the pH of the solution with the help of pH meter. Titrate the solution against 0.01 N Sodium hydroxide using Topfer's reagent as an indicator. Titrate to the endpoint when the solution turns to orange color. Note the volume of sodium hydroxide which corresponds to the free acidity. Titrate further till the solution regains pink color. Note the total volume of sodium hydroxide which corresponds to the total acidity. Acidity (mEq/1/100g) can be expressed as

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1} \text{MEq/1/100g}$$

Compare the gastric volume, acidity and ulcer index of control, STD, Ethanol extract treated an animal.

**Statistical analysis**

The results are analyzed by one way ANOVA followed by Dunnet's test and p-value <0.05 and p<0.01 was considered significant.

## 4. RESULTS

**Effect of the REIT on Pylorus ligation induced gastric ulceration**

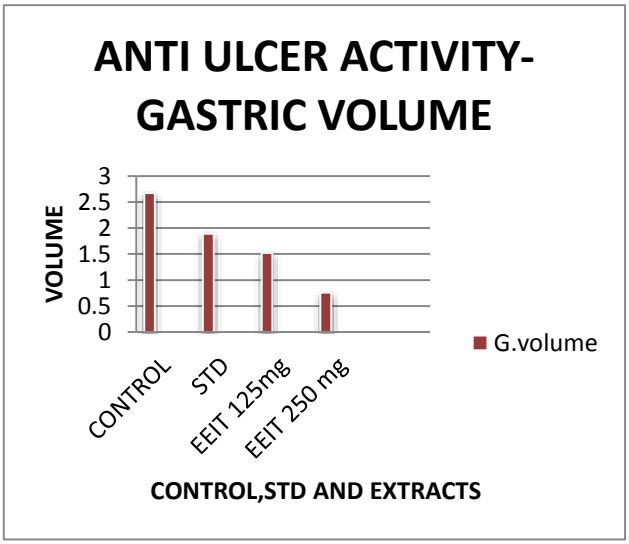
In pylorus ligated rats, the volume of gastric content, pH, total acidity, ulcer score, ulcer index are shown in Table -1 In EEIT treated groups, the volume of acid secretion, total acidity, ulcer score, and ulcer index was decreased and pH of the gastric juice was increased compared to ulcer control group. The effects of ethanol extract of *I.tinctoria* on acid parameters showed significant (p<0.01and p<0.05) effect at 125, 250 mg/kg doses compared to ulcer control animals. Ethanol extract of *EEIT* at 125 mg/kg decreased the gastric volume 1.52±0.15, total acidity 36.51±6.22, ulcer score 3.2±0.67, ulcer index 0.26±0.022 and increased gastric pH 2.24±0.49 as compared to control. Ethanol extract of EEIT at 250 mg/kg decreased the gastric volume 0.76±0.25, total acidity 30.67±6.43, ulcer score 2.33±0.76, ulcer index 0.14±0.025, and increased the gastric pH 2.83±0.59 as compared to control and 125 mg/kg extract of EEIT. This result revealed that EEIT at 250 mg/kg has more potent antiulcer activity than 125 mg/kg of extract.

**Table 1: Effect of the EEIT on Pylorus ligation induced gastric ulceration in rats**

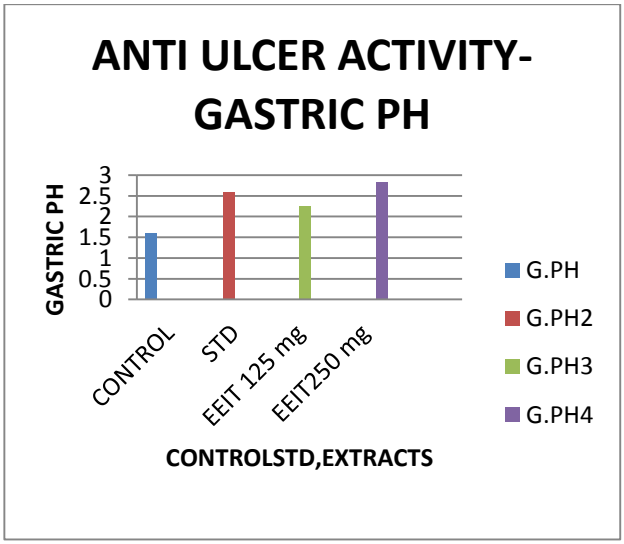
| Treatment | Dose | Gastric volume | Gastric pH | Total acidity (mEq/1/100g) | Ulcer score | Ulcer index |
|---|---|---|---|---|---|---|
| Control | 100ml/kg | 2.67±0.15 | 1.60±0.15 | 47.02±4.36 | 4.69±0.35 | 0.73±0.01 |
| STD | 30mg/kg | 1.89±0.02** | 2.60±0.35* | 21.33±6.11** | 0.89±0.03** | 0.12±0.001** |
| EEIT | 125mg/kg | 1.52±0.15** | 2.24±0.49* | 36.51±6.22* | 3,2±0.67** | 0.26±0.022** |
| EEIT | 250mg/kg | 0.76±0.25** | 2.83±0.59* | 30.67±6.43* | 2.33±0.76** | 0.14±0.025** |

Values are mean± S.D of n=6 one way ANOVA followed by Dunnet's test. P values are significant when compared with control group (* p< 0.05, **- p <0.01)

**EEIT- Ethanol extract of *Indigofera tinctoria***

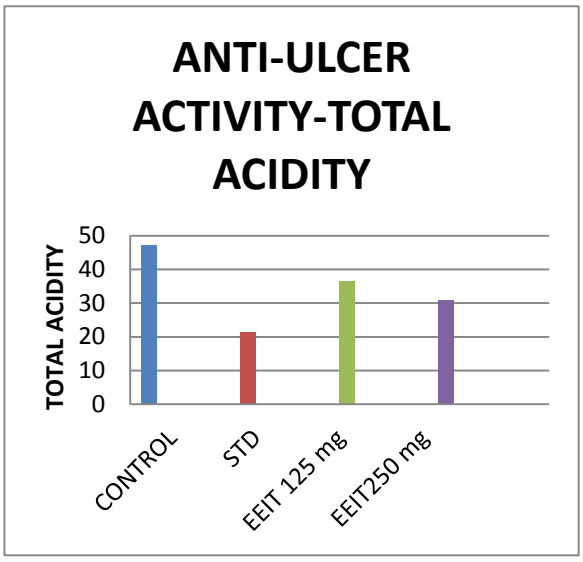



**(a)**

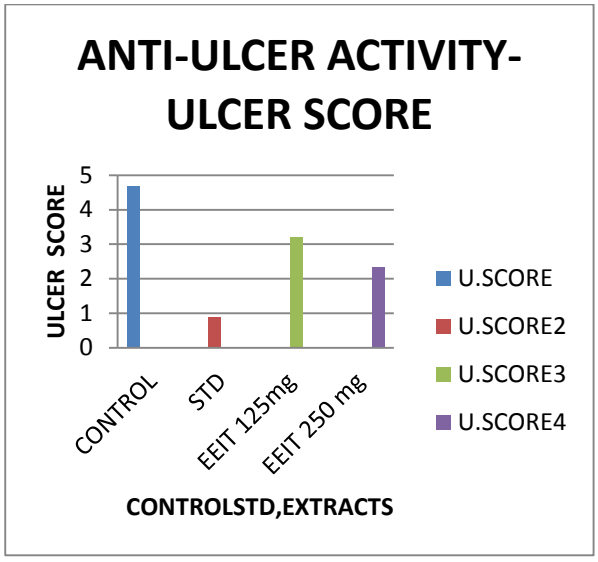



**(b)**

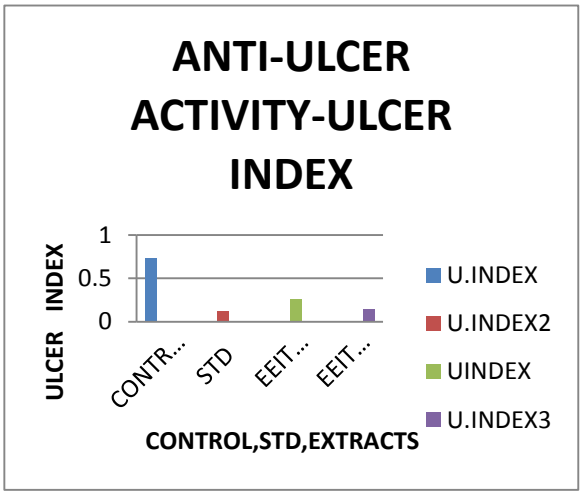
 




**(c)**




**(d)**




**CONTROL-Normal saline**
**STD-Famotidine 30mg/kg**

**(e)**

**Fig. 1 (a)-(e): Effect of the EEIT on Pylorus ligation induced gastric ulceration in rats**

## 5. DISCUSSION

The acute toxicity study revealed that the plant extract was safe in rats at a limit dose of 2000 mg/kg and that the median lethal dose ($LD_{50}$) of the extract is above 2000 mg/kg. This finding supports the work done on rats in another study.[10] The gastric ulcer is caused due to stress-induced increase in gastric acid (HCl) secretion and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid [11-13] Pylorus ligation induced ulcers are shown by auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier which resulted as upper gastrointestinal damage including lesions, ulcers and life-threatening perforation and haemorrhage. The pyloric ligation of the stomach causes accumulation of gastric acid which leads to the development of ulceration in the stomach. The agents who decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers induced by this method. Like ranitidine, famotidine acts as an anti-ulcer agent by antisecretory mechanism via inhibition of gastric secretion.[14] In the present study, Ethanolic extract of *Indigofera tinctoria* prevents the ulcer may be by the antisecretory and cytoprotective property. The preliminary phytochemical analysis of *I.tinctoria* extract showed the presence of flavonoids, terpenoids, saponin, tannins, and glycosides. The antioxidant components from many plant extracts have been extensively confirmed for their antiulcerogenic efficacy.[15] The antioxidant activity of *I.tinctoria* reported by Muthulingam et al and Renukadevi et al.[16-17] It is suggested that these active compounds would be able to stimulate prostaglandin secretion and counteract the deteriorating effects of reactive oxidants in gastrointestinal lumen.[18-19] Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit *Helicobacter pylori* growth, act as free radical scavengers, and inhibit H+/K+-ATPase.[20-21] Saponin may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation[22]. In addition, terpenoids are also reported to have potent activity against gastric ulcers.[23-24] Therefore ethanolic extract of *Indigofera tinctoria* possesses antiulcer activity, may be due to the presence of flavonoids, tannins, and terpenoids.

## 6. CONCLUSION

The present study concluded that the antiulcer activity of an Ethanolic extract of *Indigofera tinctiria* may be attributed to antisecretory and antioxidant properties. The bioactivity-guided phytochemical screening of EEIT revealed that the presence of flavonoids, tannins, saponin, and terpenoids, which may be responsible for the anti-ulcer effect and can be further fractionated and investigated for their role and utility in any of the anti-ulcer mechanisms.

## 7. ACKNOWLEDGMENT

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