



Phytochemical screening and *In vivo* anti-ulcer activity of Ethanolic extract of *Heliotropium indicum* L

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Abstract:

The phytochemical compounds and anti-ulcer activity of leaves and root extracts of *Heliotropium indicum* Linn. The preliminary phytochemical screening was performed by *in vitro* method and anti-ulcer activity was conducted by *in vivo* method. The phytochemical analysis revealed the presence of alkaloids, carbohydrates and glycosides, phytosterols, fixed oils and fats, phenolic compounds and tannins, flavonoids, terpenoids, proteins and amino acids. The ethanolic extract of *Heliotropium indicum* leaf and root was evaluated for its anti-ulcer activity against pylorous ligation induced gastric ulcer in rats. It was found that significant reduction in gastric volume, free acidity, total acidity and ulcer index. The present study concluded that the *H. indicum* plant extract have various bioactive compounds and anti-ulcer activity in animal models against the drug induced gastric ulcer.

Keywords: Phytochemical; Anti-ulcer; *Heliotropium indicum*; Pylorous ligation

INTRODUCTION

Ulcers are thought to be a result of an imbalance between "aggressive and defensive factors". It is widely accepted that pathogenesis of ulcer is complex and increased acid secretion, pepsin activity and reduced mucus and bicarbonate secretion, enhanced contractility of the gastric wall and reduced gastric mucosal blood flow represent some of the established pathogenic factors of gastric ulceration [1]. Considering the several side effects associated with the use of modern medicine in India, attempts were being made to look for suitable anti ulcerogenic plant products as a better alternative for the treatment of ulcer. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in

traditional medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts has been traced to the occurrence of natural products with medicinal properties. Plants, as sources of diverse range of bioactive molecules, have continued to play a dominant role in the maintenance of human health since ancient times [2]. Over 50% of all modern clinical drugs are of natural product origin [3] and natural products play an important role in drug development programs in the pharmaceutical industry [4].

Different kinds of plants are used in various forms of ulceration, anti periodic in fever, heumatism, tonic, beneficial in chronic dysentery, headache, blood complaints, cardiogenic, aphrodisiac, anthelmintic; good for scabies and expectorant[5]. The effects of plant extract on bacteria have been studied by a very large

number of researchers in different parts of the world [6]. Much work has been done on ethanomedical plants in India [7]. Interest in a large number of traditional natural products has been increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents [8]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products [9].

However, there is a need for isolation, characterization, determination, of bioactivity of the plant compound for its pharmaceutical application. In order to develop and to establish the safety and efficacy level of a new drug, toxicity studies are very essential and no drug is used clinically without its clinical trial as well as toxicity studies. Hence, the current study was undertaken to evaluate the anti-ulcer activity of *Heliotropium indicum* L extract by pylorous ligation induced gastric ulcer, in rats.

MATERIALS AND METHODS

Plant collection

Fresh plants of *H. indicum* L belongs to the family Boraginaceae was collected from Thanjavur District, Tamilnadu State, India and identified by the taxonomist and a special key given in 'The Flora of Presidency of Madras'. A voucher sample of the plant has been prepared and deposited.

Preparation of extracts

The leaf and root of *H. indicum* L washed with sterile distilled water. After, the leaves were shade dried for 15 days and pulverized to fine powder by using Pestle and Mortar. Plant powder was filled in

the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 hrs. All the extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further analysis.

Phytochemical screening

The freshly prepared plant extracts were qualitatively analyzed for the presence of phytochemical constituents.

Experimental animals

The ulcer study was carried out under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA/265). Albino rats of either sex weighing about 100 – 130gms were divided into groups of six each. The animals were deprived of food for 24 hours before the commencement of the experiment, but water was allowed *adlibitum*. The drugs were given orally 2 hours prior to pylorus ligation, which was carried out according to the standard technique [10].

Antiulcer Activity

The animals were divided into five groups. The group I rats were used as the normal control while the second group of rats were used as experimental untreated control. Group III treated with *H. indicum* leaf extract (100mg/kg). While group IV treated with *H. indicum* root extract (100mg/kg) and group V treated with famotidine a standard drug. For ulcer induction 5% w/v acacia mucilage was used as a vehicle at a dose of 5 ml/kg. The solvent control received equal volume of acacia mucilage. The animals were sacrificed 6 hours after pylorus ligation. The gastric juice was collected, centrifuged and its pH and volume were measured.

Total Acidity

A volume of 2 ml diluted gastric juice was titrated with 0.01N NaOH run from a micro burette using

phenolphthalein as indicator and the acidity was expressed as mg HCL/100g. body weight of rat^[12].

Free Acidity

It was determined in similar manner using topfer's reagent as indicator and sodium hydroxide was run until canary yellow color was observed^[11].

Ulcer index

Ulceration in rats was induced by standard method^[12]. On the fourth day pylorus part was ligated following 36 h fasting^[10]. The percentage inhibition of ulcer was determined^[13]. Mean ulcer score for each animal was expressed as ulcer index.

The percentage of ulcer inhibition was determined as follows:

$$\text{Inhibition of ulcer (\%)} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

RESULTS AND DISCUSSION

The results of preliminary phytochemical studies confirm the presence of alkaloids, carbohydrates and glycosides, phytosterols, fixed oils and fats, phenolic compounds and tannins, flavonoids, terpenoids, proteins and amino acids in ethanolic extracts of *H. indicum*. Plants produce a diverse range of bioactive molecules making them rich source of different source of medicines. Nowadays plant products are more popular than the synthetic drugs due to its low toxicity and

effectiveness. Flavonoids exhibit several biological effects like anti-inflammatory, hepatoprotective and antiulcer actions. Some recent reports have been indicated that many flavonoids possess antiulcerogenic activity such as quercetin, kampferol and rutin. In the present study was confirmed that *H.indicum* plant extracts also possess flavonoids.

The effect of alcoholic extract of *H.indicum* leaf and *H.indicum* root on pylorus ligated induced ulcer model was analyzed (Table: 1). Ulceration due to oral administration of acacia gum was shown in the stomach section of animal models. The gastric damage as thick red lines and lesions as red areas were observed in the stomach. It was significant to note that increase the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer control rats compared to untreated control rats. The severity in terms of volume of gastric juice, total acidity and free acidity showed decreased in leaf extract treated animals when compared to those in groups of root extract treated and standard drug treated animals. The decrease in the ulcer index in the *H.indicum* leaf and root extract treated groups were an indication of the ulcer curative nature of that plant without side effect. The present results also concordant with earlier report^[14].

Table 1: The effect of *H. indicum* leaf and root (100 mg/kg) and Famotidine (20mg/kg) on gastric ulcers

S. No	Groups	Volume of Gastric juice (ml/100g)	pH	Total Acidity (mEq/l)	Free Acidity (mEq/l)	Ulcer Lesion (mm in length)	Ulcer Index
1	Normal control	0.4±0.03	4.2±0.16	30.1±1.0	15.9±0.9	--	--
2	Ulcer control	2.8 ± 0.04	1.3±0.07	98 ± 7.3	77 ± 6.3	35.4 ± 3.2	2.82±0.05
3	<i>H. indicum</i> leaf (100 mg/kg)	0.61** ± 0.03	3.5*±0.25	38.3** ± 2.8	33.8** ± 1.8	15.8* ± 1.8	1.09±0.01
4	<i>H. indicum</i> root (100 mg/kg)	0.78* ± 0.03	3.8**±0.10	47.2* ± 0.0	33.2* ± 0.1	21.5* ± 1.0	1.32±0.08
5	Famotidine (20mg/kg)	0.50**± 0.02	4.10**±0.18	29.3** ± 1.0	16.2** ± 0.9	10.5** ± 0.8	1.00±0.02

*P< 0.01 vs ulcer control

**P< 0.001vs ulcer control by student ' t ' test

Values are ± S.E.M

The increase in volume in the ulcer control rats undoubtedly due to increased production of hydrochloric acid was an evident from the total acidity and decrease pH value of gastric juice. Inauen *et al*,^[15] were reported that inhibition of acid secretion accelerated ulcer healing. The decrease in volume of the gastric juice and concomitant decrease in the acidity and increase in pH, proving the anti-ulcer activity of *H.indicum* leaf and *H.indicum* root. But significant reduction in ulcer index was observed more in *H.indicum* leaf than the root extract. Already several scientific papers have been published related to anti-ulcer activity from medicinal plants but still numbers of plants have been screened for their efficacy ^[16]. Preliminary phytochemical investigations of the ethanolic extract of *H.indicum* showed that positive test for Liberman burchard test (sterols) and the extract treated with tin and thionyl chloride appears pink color indicates the presence of terpenoids, hence the anti-ulcer activity of *H.indicum* in this experimental model may be due to the terpenoids and steroids. This interesting observation indicates that *H.indicum* extract can be a potential source for the treatment of ulcer.

CONCLUSION

On the basis of aforesaid studies, it was concluded that alcoholic extract of both *H.indicum* leaf and *H.indicum* root provides significant anti-ulcer activity and possess various bioactive compounds. Further studies will be needed for the structural elucidation of extracted bioactive compound.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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