

## Investigation of poly-herbal aqueous extract for potential anti-ulcer activity

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### Abstract

The aqueous polyherbal extract of betel, clove, fennel and black catechu was evaluated for gastro-protective (antiulcer) activity in rats using the aspirin and ethanol induced ulcer models. Efficacy was assessed by determination of ulcer index and percentage of ulcer protection. Antioxidant activity of extract was evaluated by DPPH free radical scavenging procedure. Oral administration of the aqueous extract (250 mg/kg) and (500 mg/kg) showed dose dependent antiulcer activity and protected gastric lesions by about 65 to 75% respectively compared to standard drug Omeprazole (98%). The findings suggest that the polyherbal extract have significant gastro-protective activity.

**Key words:** Polyherb, Aspirin, Ethanol, Omeprazole, Ulcer.

### 1. Introduction

Peptic ulcer is a chronic and recurrent disease and is the most predominant of the serious gastrointestinal diseases. It is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice<sup>1</sup>. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors<sup>2</sup>. The etiology of gastro-duodenal ulcers is greatly influenced by various causative and defensive factors like acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermal growth factors<sup>3</sup>. Some other factors such as improper and inadequate dietary habits, irregular routine, excessive intake of drugs like non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by *Helicobacter pylori* are also responsible for the development of peptic ulcer<sup>4</sup>.

By Considering Several side effects (arrhythmia's, impotence, gynecomastia and haematopoeitic changes) of modern antiulcer medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer<sup>5</sup>. In spite of the progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new antiulcer compounds for development as pharmaceutical entities or alternatively as simple dietary adjuncts to existing therapies<sup>6</sup>. Naturally occurring herbs betel leaf (*Piper betle*)<sup>7</sup>, clove (*Eugenia caryophyllus*), fennel (*Foeniculum vulgare*)<sup>8</sup> and black catechu (*Acacia catechu*)<sup>9</sup> were selected because of traditional value and abundant availability in the household and local market. As they are known to act on digestive system and already having mild anti-ulcer activity these drugs were selected for evaluating antiulcer activity in combination.

### 2. Materials

The herbal drug constituents, betel leaf (*Piper betle*), clove (*Eugenia caryophyllus*), fennel (*Foeniculum vulgare*), and black catechu (*Acacia catechu*) were purchased from authorized herbal store from local market, Hyderabad. Standard anti-ulcer drug Omeprazole was obtained as gift sample from Dr. Reddy's lab, Hyderabad. All other chemicals used were of AR grade and purchased from reputed manufacturer like Ranchem and Sigma.

### 3. Methods

**3.1 Extraction:** All the dried herbs were coarsely powdered. All the powdered herbs were weighed (1 kg), mixed thoroughly and transferred into an extraction column and washed with ether to remove fatty substances. Then sufficient amount of water was added and extraction was carried out. The extract was concentrated and dried in rotary vacuum evaporator.

**3.2 Experimental Animals:** Adult male albino rats (150-200 g) of Wistar strain and albino mice (20-30 g) were used in the study. The animals were procured from NIN. The animals were acclimatized for 10day's under standard husbandry conditions, room temperature ( $27 \pm 3^{\circ}\text{C}$ ), relative humidity ( $65 \pm 10\%$ ) and 12h light/dark cycle. They were allowed free access to standard dry pelleted diet and water under hygienic conditions. Five rats were used for each group in anti-ulcer study.

**3.3 Preliminary Phytochemical Screening<sup>10</sup>:** The aqueous extract of polyherb was analyzed for the presence of various constituents. Different tests were performed on aqueous extract of polyherb for the evaluation of presence of alkaloids (Dragendorff), carbohydrates (Molisch), glycosides (Borntrager's test), saponins, Phytosterols and steroids (Salkowski), flavonoids (Shinoda), tannins (Gelatin test) and phenolic compounds (Ferric chloride) and Proteins (Ninhydrin).

**3.4 Evaluation of Antiulcer Activity**

**3.4.1 Evaluation by Aspirin Induced Ulcer Model:<sup>11</sup>** Animals were divided into 5 groups each containing 6 animals. All groups were kept fasting for 36 hrs. Group 1 served as a negative control received aspirin at the dose of 200mg/kg body weight, group 2 served as positive control and received Omeprazole at the dose of 30mg/kg body weight and the animals of group 3 received the aqueous extract of polyherb at the dose of 500 mg/kg body weight and the group 4 received the aqueous extract of polyherb at the dose of 250mg/kg body weight. Group 5 animals were control group and received only water. Aspirin was administered orally to the animals after one hour. After 6 hrs animals were sacrificed and the stomach was excised, cut along the greater curvature, washed carefully with 0.9% sodium chloride and the ulcers were scored.

**3.4.2 Ethanol Induced Ulcer Model:<sup>12</sup>** The similar procedure as that for aspirin was followed by replacing aspirin with Ethanol at the dose of 1ml/animal administered orally to the animals after 1 hr on the day of experiment.

**Table 1: Study Animal groups for antiulcer activity**

Treatment Group	Treatment	
	Aspirin induced Groups	Ethanol induced Groups
Group 1 Negative control group	Aspirin 200 mg/kg body weight	Ethanol 1ml/animal
Group 2 Positive control group	Omeprazole 30 mg/kg body weight and Aspirin 200 mg/kg body weight	Omeprazole 30 mg/kg body weight and Ethanol 1ml
Group 3 Aqueous polyherbal extract	500 mg/kg body weight and Aspirin 200 mg/kg body weight	500 mg/kg body weight and Ethanol 1ml
Group 4 Aqueous polyherbal extract	250 mg/kg body weight and Aspirin 200 mg/kg body weight	250 mg/kg body weight and Ethanol 1ml
Group 5	Only water	Only water

**3.4.3 Calculation of Ulcer Index<sup>13</sup>:** The ulcer index was calculated by summing up the total number of ulcers per stomach and total severity of ulcers per stomach using the following formulae

$$U_i = U_n + U_s + U_p \times 10^{-1}$$

Where U-ulcer, i -ulcer index, U<sub>n</sub>-average no. of ulcers per animal, U<sub>s</sub>-average no. of severity score, U<sub>p</sub>-percentage of animals with ulcers

The severity of ulcers was calculated as per the values given in table 2

**Table 2: A score of ulcers representing the severity**

Severity of ulcer	Ulcer score
Normal coloured stomach	0
Spot ulcer	1
Hemorrhagic streak	1.5
Deep ulcer	2
Perforations	3

The percentage of ulcer protection was determined as follows:

$$\text{Percentage inhibition} = \frac{\text{ulcer index of control} - \text{ulcer index of test}}{\text{ulcer index of control}} \times 100$$

**3.4.4 Evaluation of Scavenging Activity:<sup>14</sup>**

**3.4.4.1 Preparation of extract:** The powdered plant materials (betel, clove, fennel and black catechu) each 25 g was extracted thrice in distilled water (5.5 L;  $27^{\circ}\text{C}$ - $30^{\circ}\text{C}$ ) on shaker (Stuart Scientific Orbital Shaker, UK) for 48 hours. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate of aqueous extract obtained was quickly frozen and dried for 48 h using a vacuum dryer to give a yield of 20 g of dry extract. The resulting extract was reconstituted with distilled water to give desired concentrations used in this study.

**3.4.4.2: 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay<sup>15</sup>:** The method of Liyana-Pathiana and Shahidi was used for the determination of scavenging activity of DPPH free radical. One ml of 0.135 mM DPPH prepared in methanol was mixed with 1.0 ml of aqueous extract ranging from 0.2-0.8 mg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant extract was calculated using this equation;

Where,  $Abs_{control}$  is the absorbance of DPPH + methanol;

$Abs_{sample}$  is the absorbance of DPPH radical + sample (i.e. extract or standard).

**4. Results and Discussion**

**4.1 Preliminary phytochemical screening:** The screening results showed the presence of constituents in various extracts represented in the table 3.

**Table-3: Preliminary Phytochemical Screening**

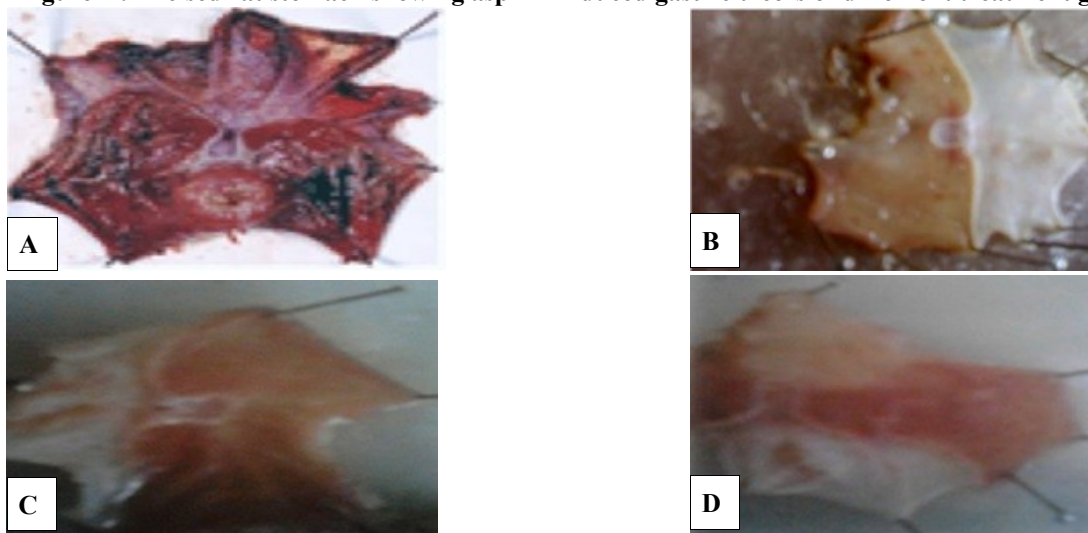
Test	Chloroform	Pet.Ether	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	-	-	+	+	+
Anthraquinones	-	-	-	-	-
Carbohydrates	-	-	+	+	+
Flavonoids	-	-	+	+	+
Glycosides	+	+	+	+	+
Protiens	-	+	+	+	-
Tannins	-	-	+	+	+
Terpenoids	+	+	+	+	+
Coumarins	-	-	+	+	+
Saponins	-	-	-	+	+

From the phyto-chemical screening aqueous extract showed the presence of flavonoids, alkaloids, tannins, terpenoids etc., therefore aqueous extract was chosen for further studies.

**4.2 Evaluation of antiulcer activity in different models<sup>16</sup>:** The study was under taken to investigate the efficacy of polyherbal extract consisting of betel leaf, clove, fennel and black catechu in the treatment of ulcer. It is well known that these drugs are rich source of antioxidant constituents. Free radicals are one of the causative agents of gastric ulcers. Free radical scavenging activity with the aqueous extracts of the above constituents (polyherbal formulation) might be responsible at least partly for antiulcer activity of these drugs.

Standard drug Omeprazole 30mg/kg was selected for both the models, polyherbal Aq. Extract doses were chosen to evaluate the ulcer protective activity in dose dependent manner i.e., 250mg and 500mg/kg b.wt. The images of excised rat stomach with various treatment groups in aspirin induced ulcer models is shown in fig. 1. From the images it can be observed that the polyherbal drug extract has significant antiulcer activity. The activity was found to be dose dependent.

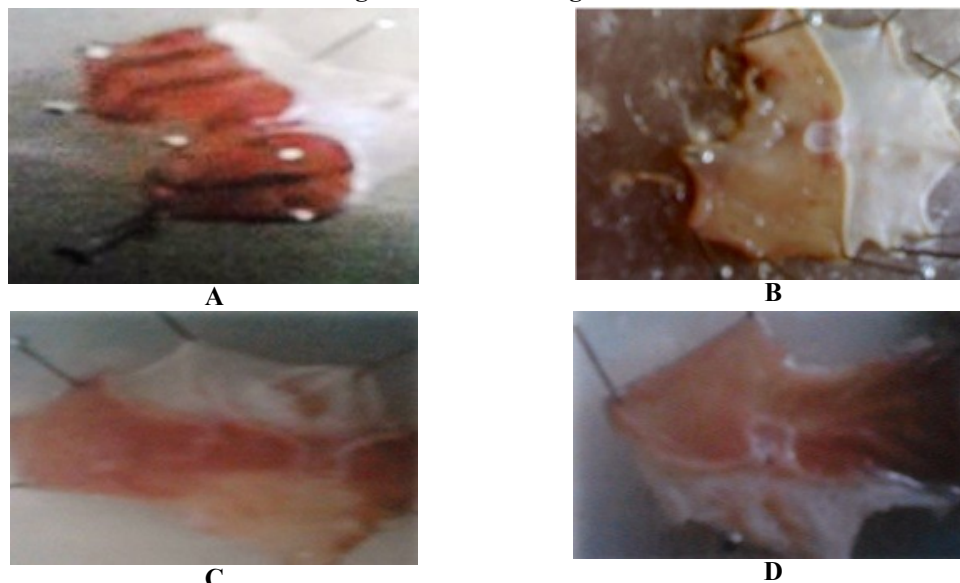
**Figure 1 : Excised rat stomach showing aspirin induced gastric ulcers of different treatment groups**



A) Negative Control (Untreated) B) Standard drug (omeprazole) C) Extract at low dose (250 mg/Kg) D) Extract at high dose (500 mg/Kg)

For further crosschecking the antiulcer activity of the polyherbal extract, activity was evaluated in ethanol induced model. The images are shown in fig 2. It can be confirmed from the images that the polyherbal extract has significant dose dependant antiulcer activity irrespective of the causing agent of ulcer.

**Figure 2 : Excised rat stomach showing Ethanol induced gastric ulcers of different treatment groups**



A) Negative Control (Untreated) B) Standard drug (omeprazole) C) Extract at low dose (250 mg/Kg) D) Extract at high dose (500 mg/Kg)

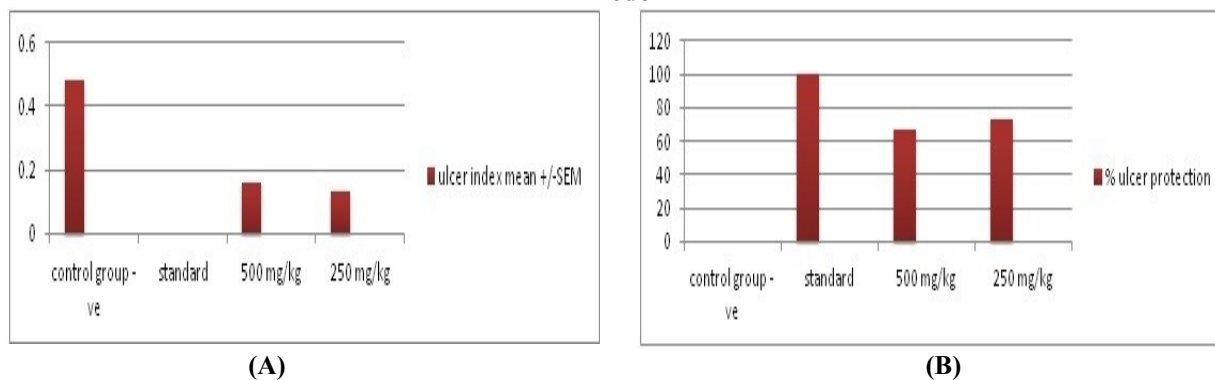
To get a quantitative estimation of antiulcer activity, ulcer index and ulcer protective ability i.e. percent ulcer protection was calculated. The obtained values of ulcer index and ulcer protection are given in Table :4. From the values it can also be confirmed that the aqueous polyherbal extract has a very good antiulcer activity.

**Table-4 Evaluation of ulcer index and percent ulcer protection in Aspirin and Ethanol induced ulcer model**

Treatment Groups	Aspirin induced ulcer model		Ethanol induced ulcer model	
	Ulcer Index	Percent Ulcer Protection	Ulcer Index	Percent Ulcer Protection
Group 1 Negative control group	0.48	0	0.74	0
Group 2 Positive control group	0	100	0.22	80
Group 3 Aqueous polyherbal extract	0.16	67	0.41	45
Group 4 Aqueous polyherbal extract	0.13	73	0.27	64

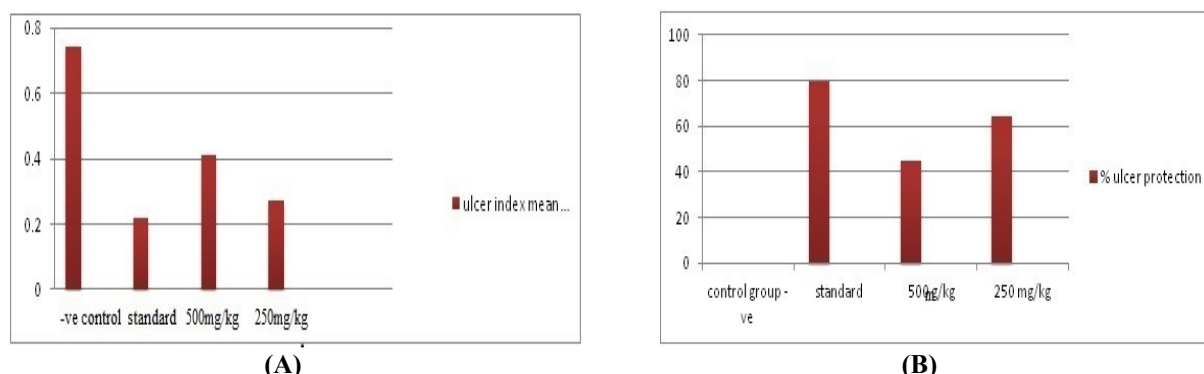
The figure 3 represent the ulcer index and % ulcer protection results of the polyherbal Aq.extract in Aspirin induced ulcer models.

**Figure 3: Obtained Ulcer index (A) and Percent Ulcer Protection (B) of different animal groups in Aspirin induced model**



Figures 4 represent the ulcer index and % ulcer protection in ethanol induced ulcer models respectively.

**Figure 4: Obtained Ulcer index (A) and Percent Ulcer Protection (B) of different animal groups in Ethanol induced model**



**4.3 Evaluation of free radical scavenging activity:** Percentage free radical scavenging activity of polyherb is good when compared to the standard BHT. The results are shown in table no 5

**Table 5: Free Radical scavenging activities of Aqueous crude polyherbal extract and BHT as standard at different concentrations.**

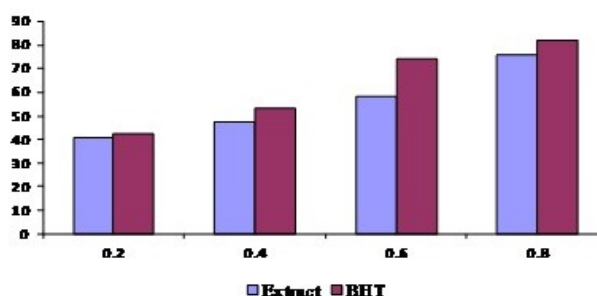
Percentage inhibition (% I) of radical scavenging of polyherbal extract				
Polyherbal Extract or BHT conc. (mg/ml)	0.2	0.4	06	0.8
DPPH	40.91(42.62)	47.63(53.00)	58.33(73.99)	75.91(82.32)

In the present study, the antioxidant activity of polyherb (betel, clove, fennel and black catechu) was determined using DPPH stable free radical scavenging assay. However, extracts of catechu and clove showed the potent activity. Betel leaf and fennel showed moderate activity.

It was observed that the aqueous extract showed 80% free radical reducing activity as compared to ascorbic acid at a conc. of 500µg/ml, 95% dpph scavenging activity as compared to ascorbic acid at a conc. of 500µg/ml. The extract was found to possess significant antiulcer activity as shown by the results-73% inhibition in aspirin induced ulcer at a lower conc. of 250mg/kg b.wt as compared to 500mg/kg b.wt which showed 67% inhibition. The aqueous extract was also found to inhibit ethanol induced ulcer- it showed 64% inhibition at a conc. of 250mg/kg b.wt.as compared to 500mg/kg b.wt. which showed 45% inhibition.

A large number of flavonoids, phenolics and tannins are known to possess strong antioxidant properties [17]. Literature review indicated the presence of these compounds in clove, catechu and betel leaf. The antioxidant activity of clove and fennel are reported<sup>18,19</sup>. The present study also confirms the same. As per ayurveda, paan which is traditional eatable contains the ingredients of our study. Antioxidant principles from natural sources provide enormous scope in correcting the imbalance between free radicals and anti-free radicals, which is the major cause of several diseases [20]. The present study shows the presence of antioxidant activity in polyherb and supports its ethnomedical use in India, which might be due to its antioxidant activity.

**Figure 7: Comparison of % radical scavenging activity of polyherb extract and standard BHT**





## 5. Conclusion and Future Studies

The above results proved the fact that the aqueous extract of polyherbal formulation is a potential antiulcer agent. The activity could be due to the presence of phytochemicals such as flavonoids, alkaloids, terpenoids etc. which are well known for their antioxidant activity. We propose here that the formulation having constituents betel leaf, fennel, clove and black catechu has a potential antiulcer activity as well as therapeutic activity in the treatment and management of diseases caused by oxidative stress. And the future studies would be in the direction of investigating the active constituent responsible for such activity and possible mechanism of action for the same.

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