

ANTIULCER ACTIVITY OF METHANOLIC EXTRACT OF *ARGYEA NERVOSA* ROOT  
BY USING ULCER INDUCED AGENTS IN RATS

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**ABSTRACT**

The anti ulcer activity of the roots of the plant *Argyrea Nervosa* was evaluated using the aspirin, Histamine induces animal model. the methanolic extract of the plant preliminary identification was done by using Phytochemical methods, after that the biochemical and animal studies were performed to identify the activity. The results discussed above it can be summarized that the plant extract possessed the antiulcer activity against the aspirin, histamine induced gastric ulceration in rats. The anti-ulcer activity of plant extract is having significant activity by using various ulcer induced agents in rats as compared to the standard drug Omeprazole.

**KEYWORDS:** *Argyrea Nervosa*, Methanolic extract, Aspirin, Histamine, anti ulcer activity.

**INTRODUCTION**

For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality. The pathophysiology of peptic ulcer has been centralized on an imbalance between aggressive and protective factors in the stomach such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. Although hospital admissions for uncomplicated peptic ulcers in developed countries had begun decrease, there was a striking rise in admissions for ulcer hemorrhage and perforation among elderly people. This increase has been attributed to the increased use of non-steroidal anti-inflammatory drugs (NSAIDs), alcoholic beverages, cigarettes and *Helicobacter pylori* infections.

Borrelli and Izzo reveal the extensive variety of chemical compounds isolated from medicinal plants with antiulcer activity. This is an important reason to investigate antiulcer effects in medicinal plants with traditional use in gastric diseases.

Peptic ulcer disease represents a serious medical problem. Approximately 500,000 new cases are reported

each year. Interestingly, those at the highest risk of contracting peptic ulcer disease are those generations born around the middle of the 20th century. Ulcer disease has become a disease predominantly affecting the older population, with the peak incidence occurring between 55 and 65 years of age. In men, duodenal ulcers were more common than **gastric** ulcers; in women, the converse was found to be true. Thirty-five percent of patients diagnosed with gastric ulcers will suffer serious complications. Although **mortality** rates from peptic ulcer disease are low, the high prevalence and the resulting pain, suffering, and expense are very costly.

**MATERIALS AND METHODS****PLANT MATERIAL**

The roots of the plant *Argyrea nervosa* were procured from **Lakshmi Medicinal Plants And Herbs Company**, Patna, and Bihar, India. The roots were cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.



Fig 4.3. Roots (Big and small) of *Argyrea nervosa*.

**PREPARATION OF METHANOLIC EXTRACT**

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of methanol for seven days. The extract was concentrated and dried using Rotary flash evaporator. It was kept in dessicator until used.

**PHARMACOLOGICAL SCREENING**

**ANIMALS USED:** Species/Common name: Swiss albino rats

- a) Age/weight/size : 150-200g  
 b) Gender : Either sex  
 c) Number used : 44  
 d) Source of animals : Sanzyme pvt. ltd, Hyderabad.(93/1999/CPCSEA)  
 e) Source of poly herb : Naturopathic dactors, Hyderabad.

Healthy Swiss albino rats of either sex, housed in animal house of DELVE LABS Pvt.Ltd (1675/RO/c/12/CPCSEA Delve Labs Private Limited,) were selected and maintained under standard laboratory conditions of light at  $23 \pm 2$  °C and  $55 \pm 5\%$  R.H. The animal housing and handling were done in accordance with CPCSEA guidelines. The experiments were conducted as per the norms of Institutional Animal Ethics Committee (IAEC). The animals were given standard rat pellet feed and tap water. After one week of acclimatization, rats were randomly selected and grouped into different groups.

**SUBSTANCES USED**

Poly Herb, Omeprazole, Aspirin. Histamine, CMC.

**CHEMICALS REQUIRED**

- ❖ Aspirin - 500mg/kg, Histamine - 100mg/kg, NaOH, Tofpers reagent, Formalin 10%, CMC 0.5%, Distilled water.

**EQUIPMENTS**

Microscope, Centrifuge, Animal weighing balance (SONIC), Mechanical stirrer(SONIC). Drug weighing balance.

**DESCRIPTION OF THE PROCEDURES USED****Preparation of Vehicle**

Dissolve 0.5g of CMC in 100 ml of water to get 0.5% CMC which is used as vehicle.

**Preparation of Standard solution**

Dissolve 0.04g of Omeprazole in 10 ml of Vehicle.

**Poly herb dose preparation**

Dissolve 500mg/kg and 1000mg/kg of Poly Herb in 1 ml of vehicle before dosing.

**5.2 METHODS****1. INDUCTION OF ULCER BY ASPIRIN<sup>[28]</sup>**

Swiss Albino rats weighing 150-200g are taken for this experiment. The rats are administered either the

appropriate vehicle or the cytoprotective drug orally 30mins prior to administration of aspirin. This process is done for 6 days and on the last day, the animals are euthanized with chloroform the stomachs are excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat and examined under a 3-fold magnifier.

**2. INDUCTION OF ULCER BY HISTAMINE**

Swiss Albino rats weighing 150-200g are taken for this experiment. The rats are administered either the appropriate vehicle or the cytoprotective drug orally 30mins prior to administration of histamine. This process is done for 6 days and on the last day, the animals are euthanized with chloroform the stomachs are excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat and examined under a 3-fold magnifier.

**5.3 EVALUATION PARAMETERS****Collection of Gastric Juice<sup>[88]</sup>**

The stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the gastric contents were removed. The gastric contents were collected in plain tubes and centrifuged at 5000 rpm for 5 min; the volume of the supernatant was expressed as ml/100 gm body weight. The mucosa was flushed with saline and observed for gastric lesions using a dissecting microscope, ulcer score was determined.

**Ulcer Scoring<sup>[28]</sup>**

After sacrificing the rat, stomach was removed and opened along the greater curvature, and washed it slowly under running tap water. Put it on the glass slide and observe under 10X magnification for ulcer. Score the ulcers as below.

0 = normal coloured stomach

0.5 = red colouration

1 = spot ulcers

1.5 = haemorrhagic streaks

2 = Ulcers  $\geq 3$  but  $\leq 5$

3 = Ulcers  $>5$

Mean ulcer score for each animal is expressed as Ulcer Index.

**Free acidity and Total acidity<sup>[28]</sup>**

Centrifuge the gastric contents at 1000 rpm for 10 min, note the volume. Pipette out 1 ml of supernatant liquid and dilute it to 10 ml with distilled water. Note the  $P^H$  of the solution with the help of  $P^H$  meter. Titrate the solution against 0.01N NaOH using toppers reagent as an indicator.(It is Dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid and total acidity in gastric fluids) Titrate to end point when the solution turns to orange colour. Note the volume of NaOH which corresponds to free acidity. Titrate further till the solution regains its pink colour. Note the total volume of NaOH which corresponds to the total acidity. Acidity (mEq/1/100 g) can be expressed as:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{Normality} \times 100}{0.1} \text{ mEq/l/100 g}$$

### Statistical analysis of data<sup>[28]</sup>

Results were expressed as mean  $\pm$  S.E.M. The statistical difference between the groups in the term of the mean rate of wound healing was calculated in terms of ANOVA mean  $\pm$  S.E.M. The difference was considered significant if  $P < 0.05$ .

The rats are divided into different groups each comprising of minimum of four rats as detailed below

**Group I** – Control rats (Aspirin +CMC)

**Group II** – Aspirin induced ulcer rats orally treated with Omeprazole (20 mg/kg b.w)

**Group III** – Aspirin induced ulcer rats orally treated with *Argyrea nervosa* the dose of 500 mg/kg

**Group IV** – Aspirin induced ulcer rats orally treated with *Argyrea nervosa* 1000mg/kg

## RESULTS

### PHARMACOLOGICAL STUDIES IN ASPIRIN INDUCED ULCERS

#### ➤ Effect of Gastric Volume

Administration of the extract and plant extract significantly decreased the gastric volume in comparison with rats treated with Omeprazole. Comparing the gastric

volume and gastric acidity, the gastric volume gets decreased, simultaneously the gastric acidity also decreased significantly.

#### ➤ Effect of Free Acidity and Total Acidity

The free acidity and total acidity was determined based on the titre values. The free acidity and total acidity of poly herb on albino rats decreased significantly in comparison with the standard group treated with omeprazole.

#### ➤ Ulcer index

The ulcer index was calculated by taking the mean ulcer score of each groups. Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-axis. The histograms of different groups were then interpolated by comparing the ulcer index of group I with group II, III and IV. It was noticed that the ulcer index of Dose group (Dose-IV) was significantly less when compared to the standard group (Group-II) treated with Omeprazole.

**Group I:** Control received only Aspirin (400 mg/kg) g

**Group II:** Dose I received plant extract on(500 mg/kg) for 6 days+ Aspirin (400 mg/kg)

**Group III:** Standard received Omeprazole (20 mg/kg)

**Group IV:** Dose II received plant extract (1000 mg/kg) for 6 days+ Aspirin (400 mg/kg)

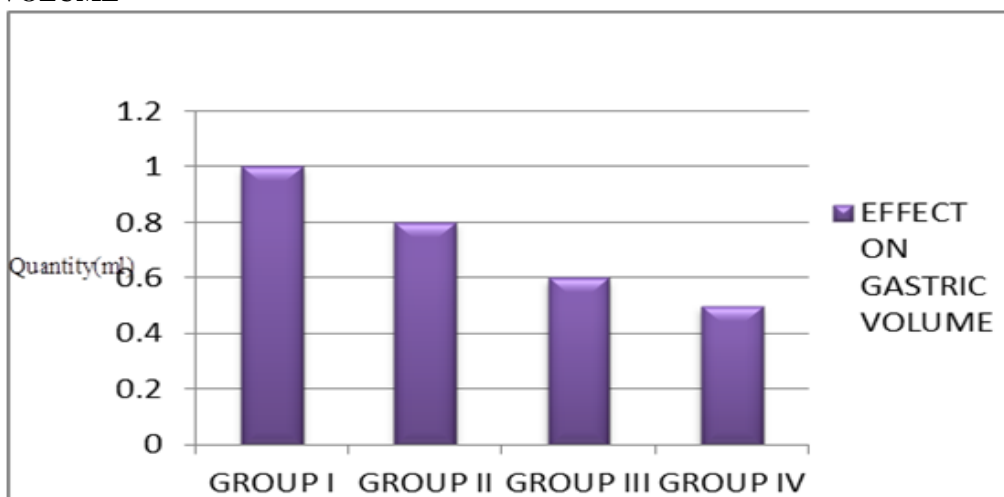
### EFFECT OF POLY HERB ON GASTRIC VOLUME IN ASPIRIN INDUCED MODEL

(Table 1)

Groups	Body wt. of rats	Drugs given	Gastric volume
GROUP I	161 $\pm$ 1.1	Aspirin + CMC	1.8 $\pm$ 0.01
GROUP II	175 $\pm$ 0.5	Omeprazole + Aspirin	1 $\pm$ 0.1
GROUP III	170 $\pm$ 0.5	plant extract (500mg/kg)+ Aspirin	1.1 $\pm$ 0.03
GROUP IV	171 $\pm$ 1.1	plant extract (1000mg/kg) + Aspirin	0.83 $\pm$ 0.02

Values are expressed in terms of mean  $\pm$  SEM of 4 rats (ANOVA), P values: \*\*< 0.001 - Highly significant, \* <0.05 - Significant, N S: Non Significant.

### EFFECT OF POLY HERB ON GASTRIC VOLUME IN ASPIRIN INDUCED MODEL GASTRIC VOLUME



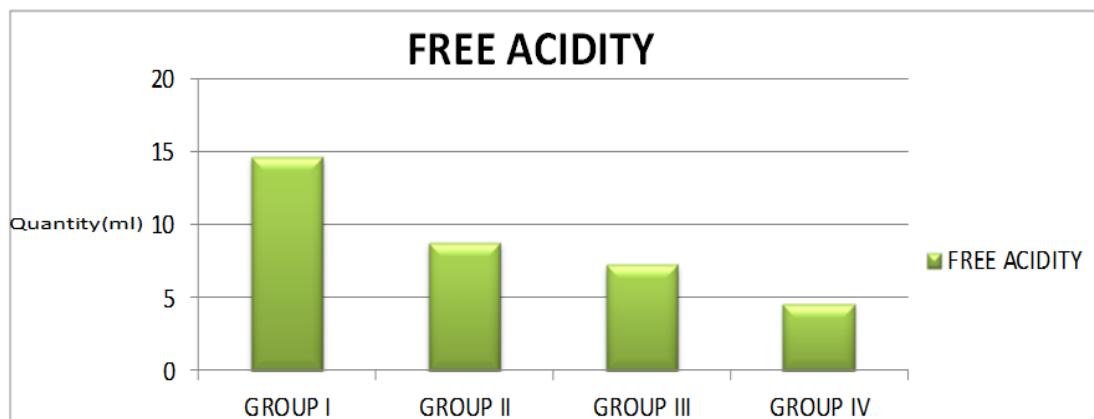
Graph 1

### EFFECT OF POLY HERB ON FREE ACIDITY AND TOTAL ACIDITY IN ASPIRIN INDUCED MODEL (Table 2)

Groups	Body wt. of rats	Drugs given	Free Acidity	Total Acidity
GROUP I	161 ± 1.1	Aspirin + CMC	14.05±0.02	29± 0.01
GROUP II	175 ±0.5	Omeprazole + Aspirin	9 ± 0.01	16 ± 0.02
GROUP III	170 ± 0.5	plant extract (500mg/kg)+ Aspirin	7 ± 0.04	13± 0.01
GROUP IV	171 ± 1.1	plant extract (1000mg/kg) + Aspirin	4± 0.08	9± 0.04

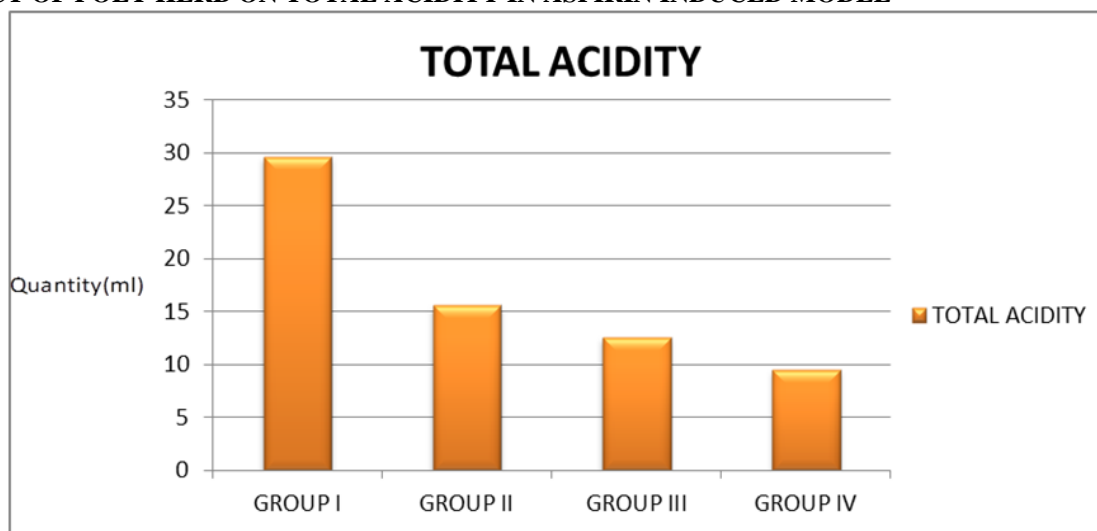
Values are expressed in terms of mean ± SEM of 4 rats (ANOVA).

### EFFECT OF POLY HERB FREE ACIDITY IN ASPIRIN INDUCED



Graph 2

### EFFECT OF POLY HERB ON TOTAL ACIDITY IN ASPIRIN INDUCED MODEL



Graph 3

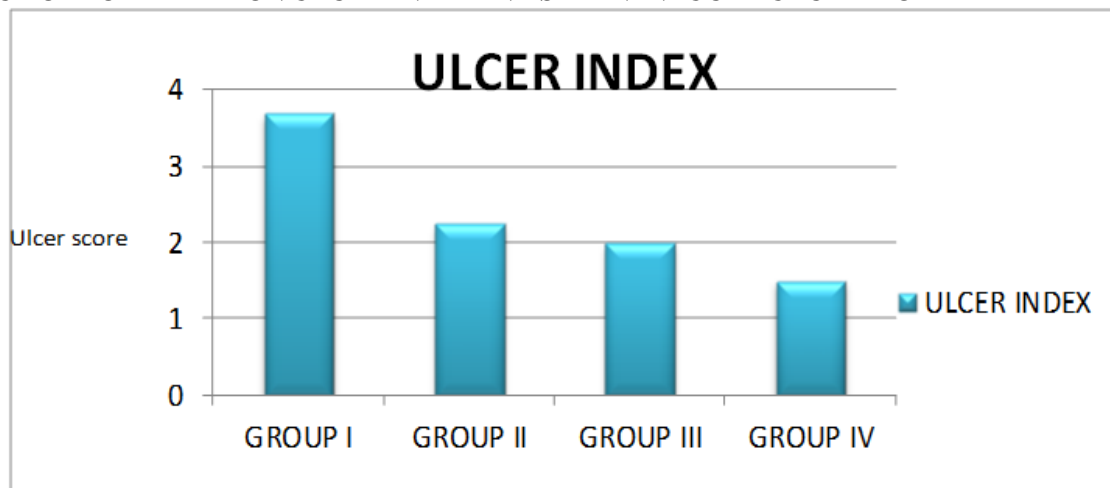
### EFFECT OF POLY HERB ON ULCER INDEX AND PERCENTAGE PROTECTION IN ASPIRIN INDUCED ULCER MODEL

(Table 3)

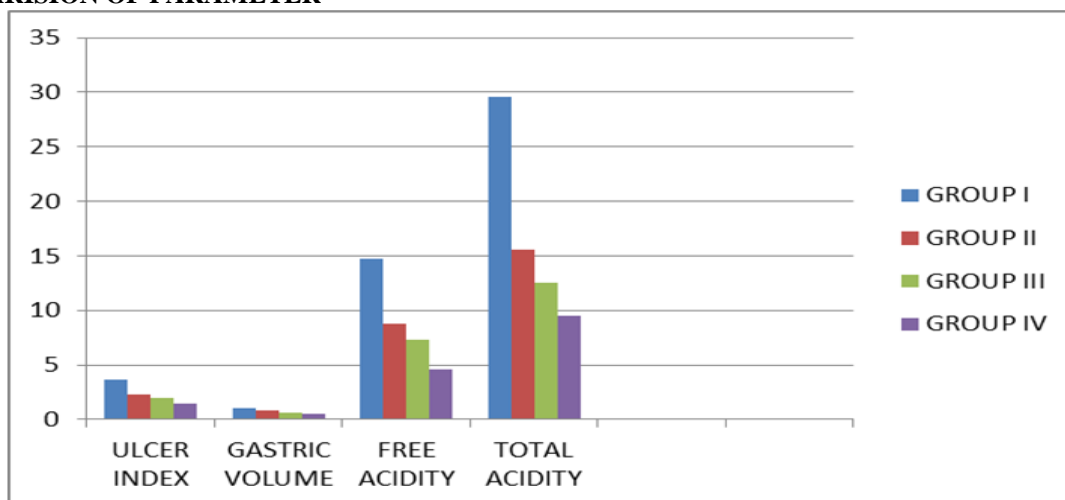
Groups	Body wt of rats	Drugs given	Ulcer index	% Protection
GROUP I	161 ± 1.1	Aspirin + CMC	3.8±0.64	-
GROUP II	175 ±0.5	Omeprazole + Aspirin	2.2±0.6*	97
GROUP III	170 ± 0.5	plant extract (500mg/kg)+ aspirin	2±0.5*	98.2
GROUP IV	171 ± 1.1	plant extract (1000mg/kg) + Aspirin	1.5±0.05**	98.8

Values are expressed in terms of mean ± SEM of 4 rats (ANOVA)

## EFFECT OF POLY HERB ON ULCER INDEX IN ASPIRIN INDUCED ULCER MODEL



Graph 4

EFFECT OF POLY HERB ON GASTRIC VOLUME FREE ACIDITY TOTAL ACIDITY AND ULCER INDEX IN ASPIRIN INDUCED MODEL  
COMPARISON OF PARAMETER

Graph 5

## 5.2 IN HISTAMINE INDUCED ULCERS

## ➤ Effect of Gastric Volume

Administration of the plant extract significantly decreased the gastric volume in comparison with rats treated with Omeprazole. Comparing the gastric volume and gastric acidity, the gastric volume gets decreased, simultaneously the gastric acidity also decreased significantly.

## ➤ Effect of Free Acidity and Total Acidity

The free acidity and total acidity was determined based on the titre values. The free acidity and total acidity of plant extract on albino rats decreased significantly in comparison with the standard group treated with omeprazole.

## ➤ Ulcer index

The ulcer index was calculated by taking the mean ulcer score of each groups. Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-

axis. The histograms of different groups were then interpolated by comparing the ulcer index of group I with group II, III and IV. It was noticed that the ulcer index of Dose group (Dose-IV) was significantly less when compared to the standard group (Group- III) treated with Omeprazole.

**Group I:** Control received only Histamine (100mg/kg)

**Group II:** Dose I received plant extract (500 mg/kg) for 6 days+ Histamine(100 mg/kg)

**Group III:** Standard received Omeprazole (20 mg/kg)

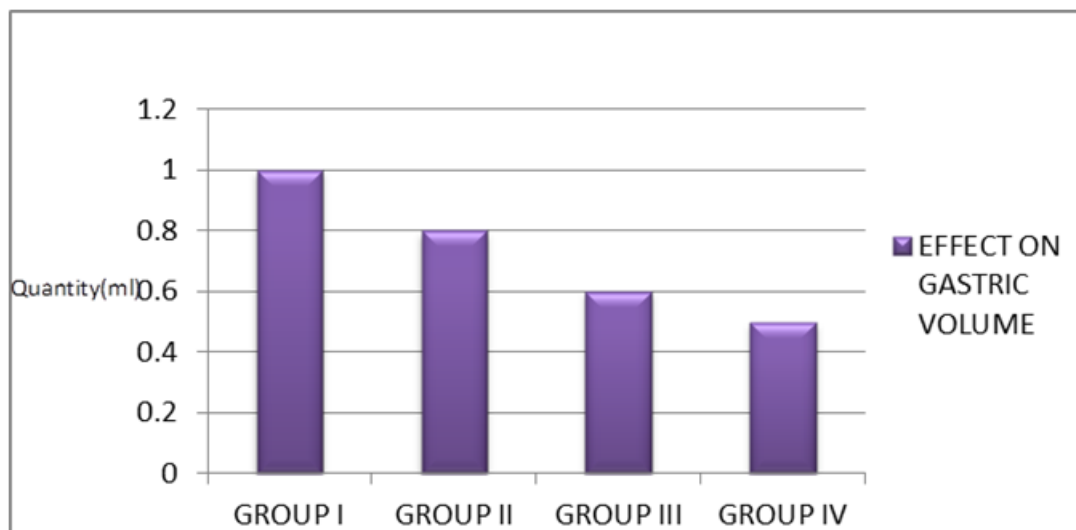
**Group IV:** Dose II received plant extract (1000 mg/kg) for 6 days+ Histamine (100 mg/kg)

### EFFECT OF POLY HERB ON GASTRIC VOLUME IN HISTAMINE INDUCED ULCER MODEL (Table 4)

Groups	Body wt. of rats	Drugs given	Gastric volume
GROUP I	176 ± 1.4	Histamine + CMC	1 ± 0.01
GROUP II	163.7 ± 0.5	Omeprazole + Histamine	0.8 ± 0.1
GROUP III	170.7 ± 0.9	plant extract (500mg/kg)+ Histamine	0.6 ± 0.02
GROUP IV	160.2 ± 0.5	plant extract (1000mg/kg) + Histamine	0.5 ± 0.02

Values are expressed in terms of mean ± SEM of 4 rats (ANOVA), P values: \*\*< 0.001 - Highly significant, \* < 0.05 - Significant, N S: Non Significant

### EFFECT OF POLY HERB ON GASTRIC VOLUME IN HISTAMINE INDUCED ULCER MODEL GASTRIC VOLUME



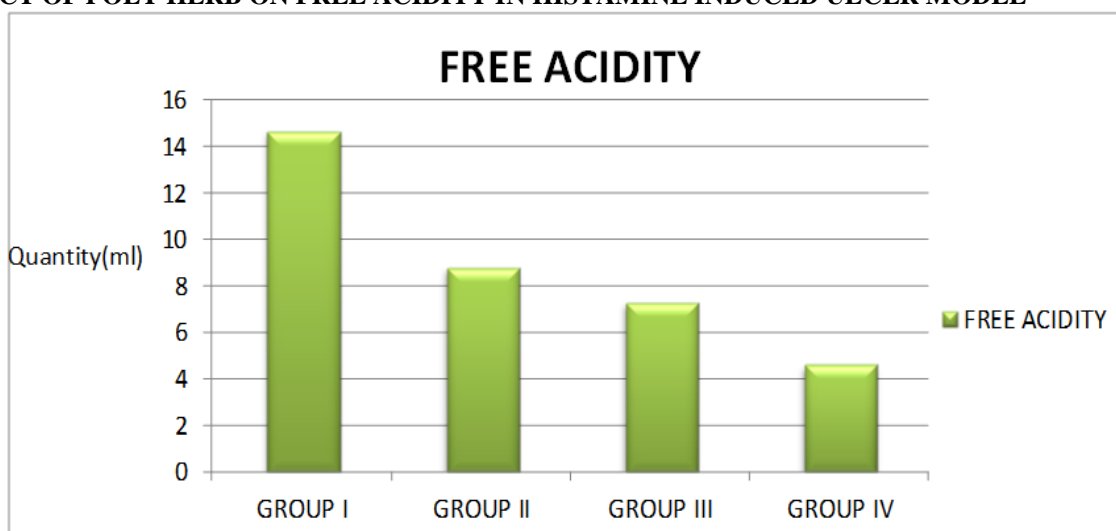
Graph 6

### EFFECT OF POLY HERB ON FREE ACIDITY AND TOTAL ACIDITY IN HISTAMINE INDUCED ULCER MODEL

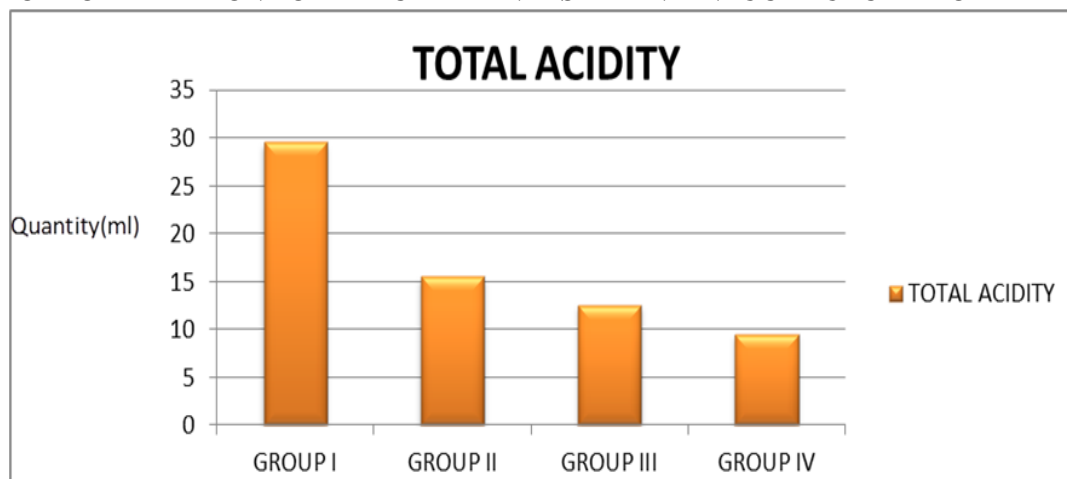
(Table 5)

Groups	Body wt. of rats	Drugs given	Free Acidity	Total Acidity
GROUP I	176 ± 1.4	Histamine + CMC	14.8±0.02	29.3±0.02
GROUP II	163.7 ± 0.5	Omeprazole + Histamine	8.8±0.03	16.4±0.04
GROUP III	170.7 ± 0.9	plant extract (500mg/kg)+ Histamine	7.3±0.02	13±0.03
GROUP IV	160.2 ± 0.5	plant extract (1000mg/kg) + Histamine	4.8±0.05	9±0.03

### EFFECT OF POLY HERB ON FREE ACIDITY IN HISTAMINE INDUCED ULCER MODEL



Graph 7

**EFFECT OF POLY HERB ON TOTAL ACIDITY IN HISTAMINE INDUCED ULCER MODEL**

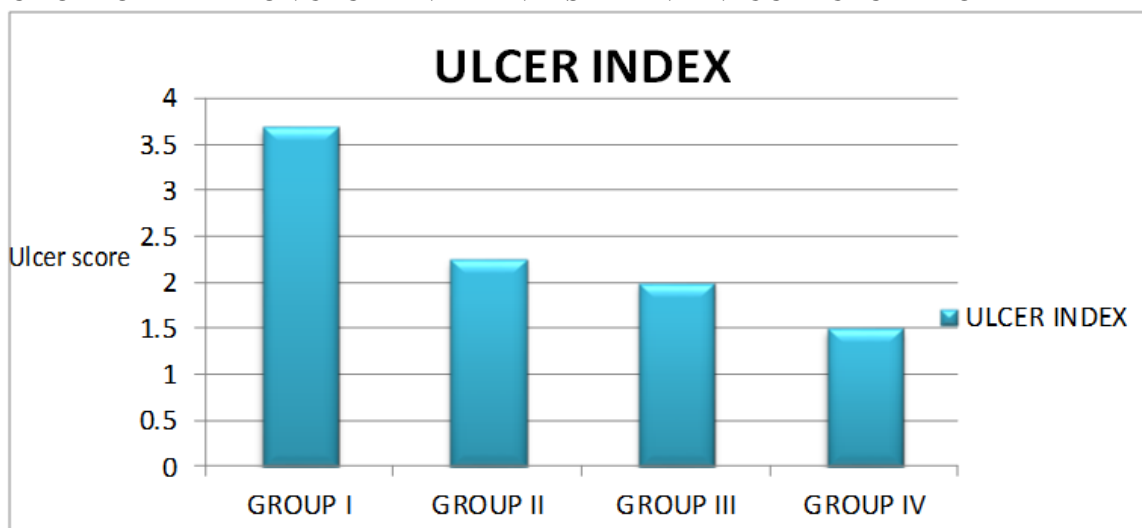
Graph 8

**EFFECT OF POLY HERB ON ULCER INDEX AND PERCENTAGE OF PROTECTION IN HISTAMINE INDUCED ULCER MODEL**

(Table 6)

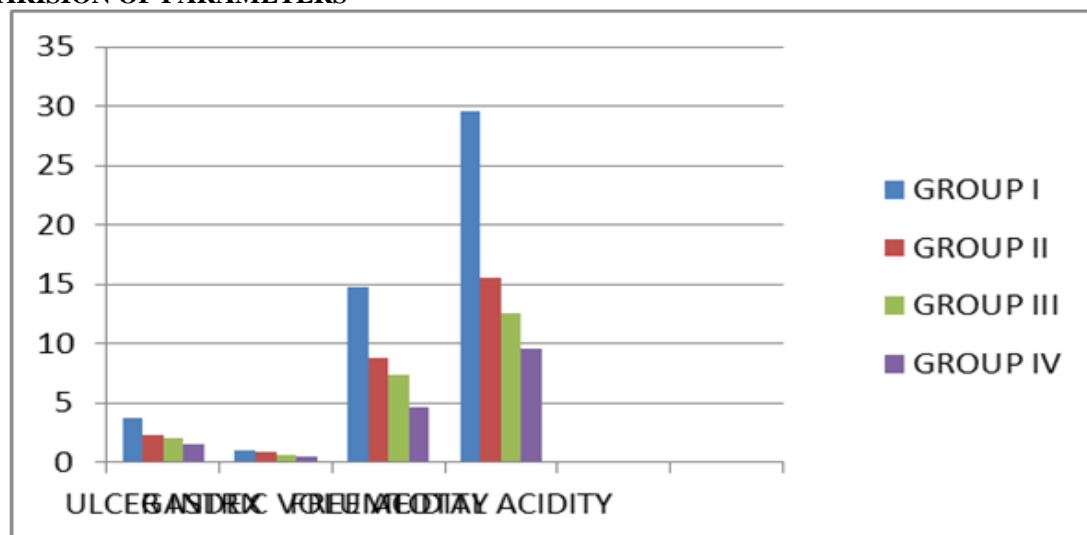
Groups	Body wt of rats	Drugs given	Ulcer index	% Protection
GROUP I	176 ± 1.4	Histamine + CMC	3.8±0.4	-
GROUP II	163.7 ±0.5	Omeprazole + Histamine	2.2±0.47**	98
GROUP III	170.7 ± 0.9	plant extract (500mg/kg)+ Histamine	2±0.4*	98.2
GROUP IV	160.2 ± 0.5	plant extract (1000mg/kg) + Histamine	1.5±0.70*	98.8

Values are expressed in terms of mean ± SEM of 4 rats (ANOVA)

**EFFECT OF POLY HERB ON ULCER INDEX IN HISTAMINE INDUCED ULCER MODEL**

Graph 9

### EFFECT OF POLY HERB ON GASTRIC VOLUME FREE ACIDITY TOTAL ACIDITY ULCER INDEX IN HISTAMINE INDUCED ULCER MODEL COMPARISION OF PARAMETERS



Graph 10

#### DISCUSSION

It is evident from the result of the present investigation that the plant extract which possesses antiulcer activity in aspirin induced, histamine induced acute ulcers. Poly Herb provided more consistent antiulcer activity.

Plant extract anti ulcerogenic activity was studied in aspirin induced gastric mucosal damage model in swiss albino rats. This model was chosen because NSAID abuse is the main exogenous cause of refractory peptic ulcer constituting 39% of the cases of peptic ulcer. NSAIDs produce a spectrum of injury to the gastro duodenal mucosa, from haemorrhages and petechiae to erosions and ulcers. Aspirin is known to inhibit PG cyclooxygenase, leading to reduced production of PGE and endothelial PGI. This causes vasoconstriction, inhibition of platelet aggregation (enhanced bleeding) and contributes to the enhanced acid secretion. It can also cause mast cell degranulation resulting in the release of histamine. Tissue damaging free radicals which are produced from the conversion of hydroperoxy to hydroxy fatty acids further contribute to cell destruction. In our study poly herb significantly reduced the ulcers induced by aspirin, histamine and ethanol results were comparable to omeprazole.

Histamine induced gastric ulcers are mediated through both enhancement of gastric acid secretion and its vasospastic action. Histamine causes vasodilatation and produces changes in the microvasculature of the mucosa which promotes bleeding of the mucosa. Histamine released from the mast cells of the gastric mucosa acts on the H<sub>2</sub> receptors on the parietal cells that are linked to the stimulation of adenylyl cyclase causing activation of cyclic AMP. Thus it increases acid secretion and results in mucosal damage. It eliminates the inhibitory influence of somatostatin & augments acid secretion. Histamine is a neurotransmitter present in discrete areas of the brain in

small amounts. The brain content of histamine is due to mast cell rather than neurons. Histamine by acting on H<sub>2</sub> receptors in the CNS produces depression. Depression reduces gastric mucosal blood flow and gastric motility which leads to mucosal damage.

Omeprazole the proton pump inhibitor play an important role in the reduction of gastric volume and total acidity and thus perform a cytoproective effect. from references it is observed that by comparing the effect of various clinical agents on healing of ulcers induced by aspirin, we observed that among different anti-secretory and cytoprotective agents.

#### CONCLUSION

From results discussed above it can be summarized that the plant extract possessed the antiulcer activity against the aspirin, histamine induced gastric ulceration in rats. The anti-ulcer activity of plant extract is having significant activity by using various ulcer induced agents in rats as compared to the standard drug Omeprazole.

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