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## PROTECTIVE ROLE OF *EQUISETUM ARVENSE* AGAINST PEPTIC ULCER IN MALE ALBINO WISTAR RATS

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### ABSTRACT

Ethnographic relevance: *Equisetum arvense* has been used in the treatment of peptic ulcer.

#### Aim of Study:

The present study was conducted to evaluate the potential of *Equisetum arvense* extract in peptic ulcers and explore its possible mechanism of action.

#### Material and Methods:

*Equisetum arvense* peptic ulcer in male albino Wistar rats was used to evaluate the peptic ulcer activity of *Equisetum arvense* extract at an oral dose of 150 mg/kg, 300 mg/kg, and 250mg/kg for 7 days. Identify Catalase, estimation of reduced glutathione (GSH), estimation of nitrate levels.

#### Results:

Significant ( $p < 0.05$ ) reduction in Catalase, estimation of reduced glutathione (GSH), estimation of nitrate levels. And peptic ulcer score by *Equisetum arvense* extract. *Equisetum arvense* extract has prevented decrease in peptic ulcer.

#### Conclusion:

Current media information suggests that *Equisetum arvense* shows protective activity in peptic ulcer, which may be due to its improved antioxidant and anti-inflammatory activity.

**Keywords:** Anti-inflammatory activity, *Equisetum arvense*, Peptic ulcer, Male Albino Wistar Rats

## INTRODUCTION

Peptic ulcers are open hollows or lesions. It is developed in the mucosa of the stomach or the duodenum i.e. the first section of the small intestine [1]. Normally coating of mucus and other chemicals they shield the stomach and duodenum from digesting themselves [2]. When protective mechanisms are disrupted so powerful digestive acids can erode into the mucosa of these organs and they cause peptic ulcers [3]. *Equisetum arvense* L. commonly known as Horsetail is a bushy perennial herb, originally native to northern hemisphere [4]. *Equisetum* species is widely distributed throughout Canada, USA except the southeast, Europe and Asia south to Turkey, Iran, the Himalayas, across China (except the south eastern part) [5], Korea and Japan *E. arvense* belongs to Equisetaceae family in the order of Equisetales that contains just only one living genus [6]. The genus *Equisetum* consists of 30 species of rush like, conspicuously jointed, perennial herbs [7].

## MATERIALS AND METHODS

### Plant Material

*Equisetum arvense* powdered (mesh size 250) have been supplied by pharmaceutical shop, Jadavji Lallubhai & Co. in Hyderabad, India [8].

### Preparation of Plant Extract

*Equisetum arvense* powder are weighed up to 40-50 percentage and soaked with water and kept maceration for 24 h for softening which leads to easier extraction later. After maceration the extract should be filtered and the resulted extract should be preserved safely.

### Experimental Animals

Animals were obtained from the Mahaveer Enterprises, Hyderabad. Adult male wistar rats,

Weights (150-200g) were used in the present study. Animals were maintained under standard

Laboratory conditions (12:12 light/darkness; at 23±10C) with standard animal diet and water

Available ad libitum. Our collage was approved by CPCSEA for conducting animal experiments with the registration number: 516/01/A/CPCSEA.

### Experimental Design

Before the experiment, the rats were acclimatized for a period of two weeks, and then rats

Were kept fasted for 18h prior to the experiment with water and libitum. Now we have randomly divided into four groups of six animals. Group I rats were (control group), Group II were (disease control), Group III were ((low dose) Group IV were (high dose), group V standard.

Table 1: Experimental design

Groups	Treatment
Group-1	Control group (CG)
Group-2	Disease control (DC)
Group-3	Low dose of Test compound
Group-4	High dose of Test compound
Group-5	standard

## Evaluation of Peptic Ulcer

### Estimation of Catalase [9]

#### 1) Tris buffer (0.25 M, pH-8.3) solution:

3.0285 g of Tris buffer was weighed and transferred to a volumetric flask and the volume made up to 100 ml with distilled water.

#### 2) Potassium Phosphate Buffer (50Mm, pH 7.0) Solution:

87mg of Dipotassium hydrogen phosphate and 680 mg potassium dihydrogen phosphate were weighed and transferred to a volumetric flask and the volume made up to 100ml with distilled water. The pH is adjusted to 7.0 by adding NaOH.

#### 3) Hydrogen Peroxide (30mM) Solution:

34µL of 30% H<sub>2</sub>O<sub>2</sub> was pipetted out and transferred to a volumetric flask and the volume made up to 10 ml with distilled water.

#### To Estimate Catalase in Tissue:

- Catalase activity was measured by the method. Tissue is homogenized

in ice-cold Tris buffer to produce a 10% (w/v) homogenate.

- Homogenate is centrifuged at 10,000rpm at 4°C for 15min. To 0.1 ml of centrifuged tissue homogenate, 1.9 ml of 50mM Phosphate buffer.
- To this mixture 0.1 ml of 30mM H<sub>2</sub>O<sub>2</sub> were added and the changes in absorbance was measured for 3 min at 240 nm at an interval of 30 sec.
- A control was prepared using 0.1ml of distilled water devoid 0.1ml of homogenate.
- One unit of the enzyme activity is defined as enzyme concentration required inhibiting the change in the absorbance by 50% in one min in the control sample.
- Activity of catalase was expressed as moles of H<sub>2</sub>O<sub>2</sub> metabolized/mg protein/min.

$$\text{Formula } \frac{\Delta \text{O. D of the sample}}{43.6 \times \text{ml of enzyme}} \times \frac{2.5}{\text{Protein in mg/ml}}$$

### Estimation of Reduced Glutathione (GSH): [10]

Preparation of reagents:

**1. Sodium Phosphate buffer (0.4M, pH-7):**

Weigh 5.68 g of disodium hydrogen phosphate, dissolve it in 100 ml of distilled water and adjust the pH to 7 with HCl.

**2. EDTA (0.8mM):** Weigh 29.78mg of EDTA and dissolved in 100ml of distilled water.

**3. Sodium Azide (10mM):** Weigh 6.5 mg of sodium azide into a 10 ml volumetric flask and dissolve with distilled water.

**4. Hydrogen peroxide (30mM):** Measure 34ul of H<sub>2</sub>O<sub>2</sub> dissolved in 10 ml of distilled water.

**5. Reduced glutathione (4mM):** Weigh 2.29 mg of GSH, transfer to a 10 ml volumetric flask and dissolve with distilled water. TCA 1%.

**6. Trichloacetic acid (10%):** Weigh 10 g of TCA into a 100 ml volumetric flask and dissolve with distilled water.

**7. Disodium hydrogen phosphate (0.3mM):** Weigh 4.26 mg of NaHPO<sub>4</sub> into a 100 ml volumetric flask and dissolve with distilled water.

**8. Trisodium citrate (1%):** Weigh 1 g of trisodium citrate (TCA), transfer it to a 100 ml volumetric flask and dissolve with distilled water. 9. 5, 51-dithio bis (2-nitrobenzoic acid) (0.04%):

Weigh 4 mg of DTNB, transfer to a 10 ml volumetric flask and dissolve with

### Procedure:

- Take 0.2 ml of tissue homogenate and 0.2 ml of 0.8 mM EDTA, 0.1 ml of sodium azide, 0.1 ml of 4 mM GSH, 0.1 ml of H<sub>2</sub>O<sub>2</sub> solution and 0.4 ml of 0.4 M phosphate buffer (pH-7).
- Incubate at 37°C for 10 minutes. Keep the tubes at room temperature and add 0.5 ml of 10% TCA and centrifuge at 2000 rpm for 10 min, adding 0.1 ml of 0.04% DTNB solution to the supernatant.
- Read the optical density at 420nm against blank
- Expressed as  $\mu\text{l moles of glutathione oxidized / min / mg protein}$ .

### Estimation of Nitrate Levels [11]

The tissue nitrite levels were estimated by using the Griess reagent method.

- To the 100mg of the sample (tissue) add 100ul of both ZnSO<sub>4</sub>(5%) and NaOH (0.3M).
- It was mixed with the sample in order to remove the proteins from the sample.
- After that centrifuge the above mixture at 6400g for a period of 15 to 20min.
- The 1.0mL of Griess reagent was added to the 20ul of the supernatant obtained after centrifugation

**Griess reagent:** 100mg of sulfanilamide and 10mg of naphthylethylene diamine hydrochloride were weighed transferred to a volumetric flask and the volume was made

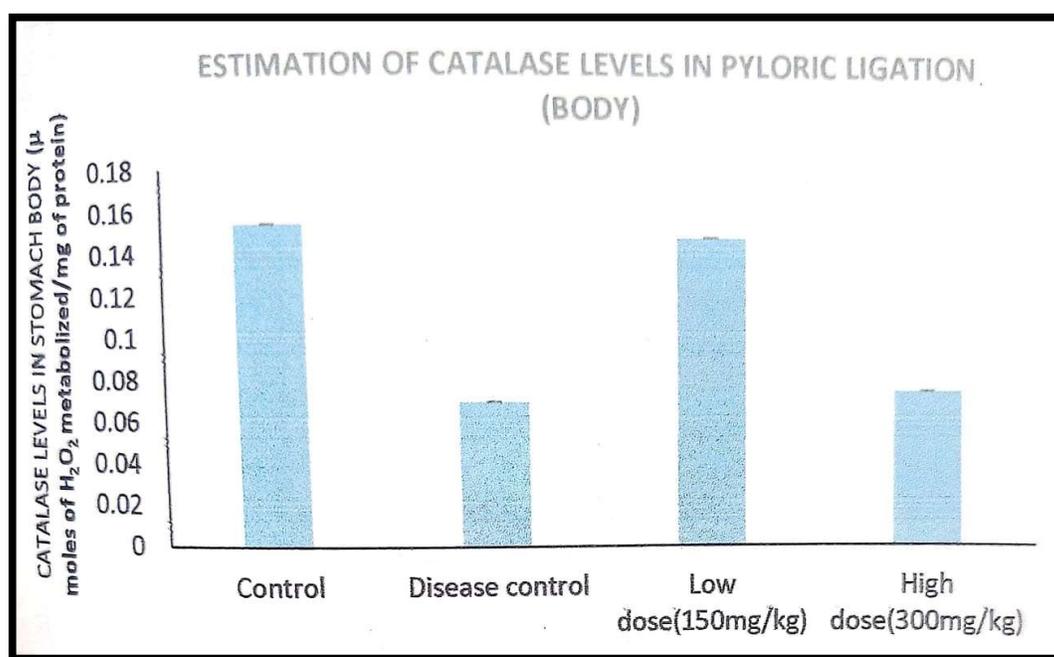
up to 10ml with 59% phosphoric acid solution. The absorbance was measured at 540nm.

**RESULTS**

Table 1: effect of AQEA of catalase levels in pyloric ligation induced ulcers in male albino wistar rats. in albino wistar rats

Groups	Estimation of catalase levels in pyloric ligation ( $\mu$ moles of $H_2O_2$ Metabolized /mg of protein						Mean $\pm$ SEM
	R1	R2	R3	R4	R5	R6	
Control	1.6	1.3	1.4	1	2	1.9	1.53 $\pm$ 0.15
Disease control	0.4	0.1	0.4	0.6	0.3	0.5	0.38 $\pm$ 0.07#
AQEA (150mg/kg)	1.2	1	1.2	1.8	1.5	1.9	1.43 $\pm$ 0.14***
AQEA (300mg/kg)	1.6	1.2	1.4	1.7	1.6	1.4	1.48 $\pm$ 0.07***

All values represented MEAN $\pm$ SEM, n=6, \*\*\*P<0.001, \*\*P<0.01, \*P<0.05 and <sup>ns</sup>P<0.05.when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett’s multiple comparison test

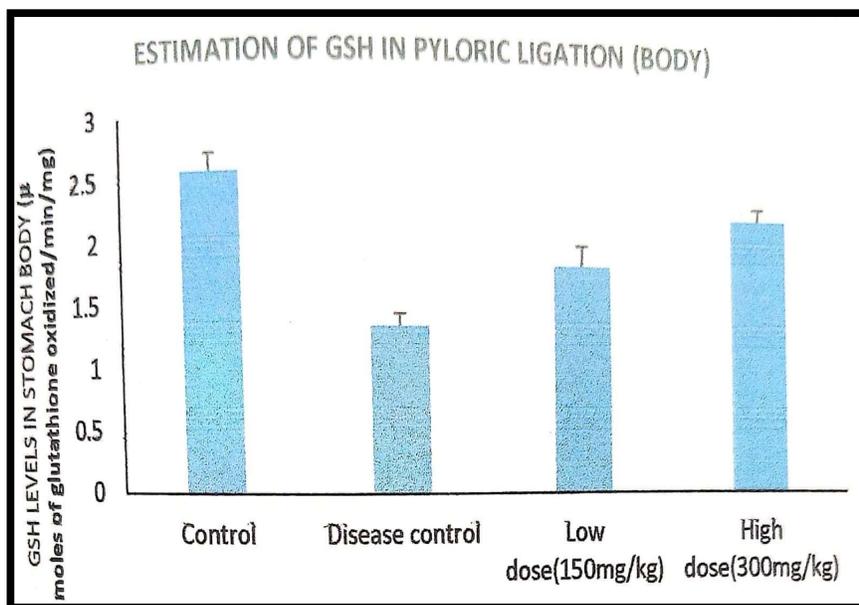


Graph 1: Effect of AQEA of catalase levels in pyloric ligation induced ulcers in male albino wistar rats. In albino wistar rats

Table 2: effect of AQEA of GSH levels in pyloric ligation induced ulcers in male albino wistar rats. in albino wistar rats

Groups	Estimation of GSH levels in pyloric ligation ( $\mu$ moles of $H_2O_2$ Metabolized /mg of protein.						Mean $\pm$ SEM
	R1	R2	R3	R4	R5	R6	
Control	2.6	2.4	2.1	3	2.9	2.4	2.56 $\pm$ 0.13
Disease control	1	1.7	1.4	1.2	1.4	1.3	1.33 $\pm$ 0.09#
AQEA (150mg/kg)	2.1	1.2	1.7	2.4	1.8	1.7	1.81 $\pm$ 0.16*
AQEA (300mg/kg)	1.9	2.3	2.1	2.5	2.1	2.4	2.21 $\pm$ 0.09***

All values represented MEAN $\pm$ SEM, n=6, \*\*\*P<0.001, \*\*P<0.01, \*P<0.05 and <sup>ns</sup>P<0.05.when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett’s multiple comparison test.

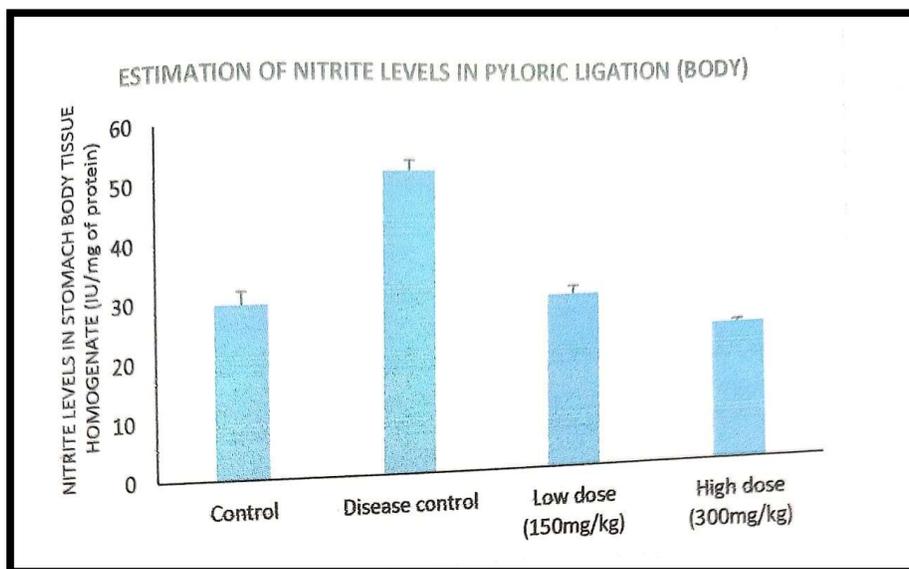


Graph 2: Effect of AQEA of GSH levels in pyloric ligation induced ulcers in male albino wistar rats. In albino wistar rats

Table 3: effect of AQEA of nitrate levels in pyloric ligation induced ulcers in male albino wistar rats. in albino wistar rats

Groups	Estimation of nitrate levels in pyloric ligation (µ moles of H <sub>2</sub> O <sub>2</sub> Metabolized /mg of protein.						Mean ± SEM
	R1	R2	R3	R4	R5	R6	
Control	24.6	21.3	33.5	24.5	36.7	29.2	29.7±2.2
Disease control	59.4	52.9	48.6	51.8	50.6	49.5	51.98±1.76#
AQEA (150mg/kg)	34.3	26.8	25.9	28.1	31.8	30.3	30.08±1.32***
AQEA (300mg/kg)	25.6	21.4	23.7	24.1	23.6	22.2	23.84 ± 0.49***

All values represented MEAN±SEM, n=6, \*\*\*P<0.001, \*\*P<0.01, \*P<0.05 and #P<0.05. when compared to Disease Control and ###p<0.001 when disease control compared with Control Group, using one-way ANOVA-Dunnett's multiple comparison test



Graph 3: Effect of AQEA of nitrate levels in pyloric ligation induced ulcers in male albino wistar rats. In albino wistar rats

## DISCUSSION

Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins (PG), and nitric oxide). The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Different classes of drugs are used in the treatment of peptic ulcer but most of these drugs exhibit serious side effects like arrhythmias, gynaecomastia, impotence, arthralgia, and hyper-gastrinemia and haemopoietic changes. Alternative approach in recent days is the research of medicaments from Ayurvedic or traditional medicinal system. Current therapeutic techniques to treat gastric ulcers are based on antisecretory drugs, whose adverse effects include the rebound effect of acid hypersecretory secretion. This has contributed to the increasing demand for natural products to treat the use of phytoconstituents in 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. According to recent studies, 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 [10]. Hence, efforts are on to find a suitable treatment from natural product sources. Various polyphenolic compounds have been reported for their anti-ulcerogenic activity with a good level of gastric protection. *Equisetum arvense* (AQEA) (Equisetaceae) contains alkaloids, carbohydrate, proteins and amino acids, phytosterols, saponins, tannin flavonoids and sterols, ascorbic acid, silicic acid, phenol triterpenoids [11]. The phenolics compounds such as silicic acid, tartaric gastric ulcers with fewer adverse effects. Acid Methyl esters of protocatechuic, caffeic acids isoquercitrin, apigenin and kaempferol were responsible for the pharmacological actions of *Equisetum arvense*. Besides their action as gastroprotective, these phenolic compounds can be an alternative for the treatment of gastric ulcers. Therefore, considering the important role of polyphenolic compounds in the prevention or reduction of gastric lesions. The present study was designed to evaluate the effect of *Equisetum arvense* for antiulcer activity using different methods like catalase, GSH & nitrate level. These 3 models are related in the present study involving, depletion of gastric wall, mucosal damage induced by non-steroid anti-inflammatory drugs and free radical

production. Pyloric ligation-induced ulcer model is an important method for the measurement of mean ulcer index in ulcer genesis. Gastric ulceration in this method may be the stress-induced secretion of HCl in excess amounts from the parietal cells and auto digestion of mucosa by the gastric juice. In this study, catalase, GSH and nitrate levels were observed in rats after pyloric ligation, these findings revealed that AQEA mainly suppressed the induced gastric lesions by inhibiting oxidative stress.

### CONCLUSION

The AQEA produced a significant decrease in the gastric volume, free acidity and total acidity compared to the disease control group, indicating an anti-secretory mechanism. The AQEA in all doses showed a significant reduction in the ulcer index compared to the control group, indicating gastro protective effect. The AQEA showed an increase in pH and a decrease in H<sup>+</sup> concentration in the gastric contents by decreasing acid secretion. The AQEA showed reduced levels of catalase, GSH, nitrite, in peptic ulcer induced rats. The AQEA showed significant elevation of GSH in peptic ulcer induced rats. The antiulcer activity of *Equisetum arvense* might be due to their antisecretory, antacid, antioxidant and anti-inflammatory activities. The pharmacological actions of *Equisetum arvense* due to the presence of chemical constituents.

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