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EQUISETUM ARVENSE PROTECTIVE EFFECT USING DIFFERENT SCREENING MODELS ALBINO WISTAR RATS AGAINST THE PEPTIC ULCER

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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. Determination of protein estimation method, estimation of superoxide dismutase, IL6 ELISA Method.

Results:

Significant ($p < 0.05$) reduction in Determination of protein estimation method, estimation of superoxide dismutase, IL6 ELISA Method. 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, peptic ulcer in male Albino Wistar Rats

INTRODUCTION

Peptic ulcer disease (PUD) is characterized by discontinuation in the inner lining of the gastrointestinal (GI) tract because of gastric acid secretion or pepsin. It extends into the muscularis propria layer of the gastric epithelium. It usually occurs in the stomach and proximal duodenum **[1]**. It may involve the lower esophagus, distal duodenum, or jejunum. Epigastric pain usually occurs within 15-30 minutes following a meal in patients with a gastric ulcer **[2]**; on the other hand, the pain with a duodenal ulcer tends to occur 2-3 hours after a meal. Today, testing for Helicobacter pylori is recommended in all patients with peptic ulcer disease. Endoscopy may be required in some patients to confirm the diagnosis, especially in those patients with sinister symptoms. Today, most patients can be managed with a proton pump inhibitor (PPI) based triple-drug therapy **[3]**.

Materials and Methods

Plant Material

Equisetum arvensepowdered (mesh size 250) have been supplied by pharmaceutical shop, Jadavji Lallubhai & Co. in Hyderabad, India **[4]**.

Preparation of Plant Extract

Equisetum arvensepowder are weighed up to40-50 percentage and soaked with water and kept maceration for 24 h for softening which leads to easier extraction later. After maceration theextract should filtered and the resulted extract should be preserved safely.

Experimental Animals

Animals were obtained from the MahaveerEnterprises, 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 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 **[5]**.

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

Determination of Protein Estimation Methods: [6]

The tissue samples were used for the estimation of all parameters. The eye ball was excised and pulls off the lens out carefully. The lens were weighed and homogenate prepared by using the TWEEN buffer pH 8.

Preparation of TWEEN buffer pH 8(100ml):

Tris 250mM -30.285gm

Nacl IM -58.5gm

EDTA 5mM -1.85gm

NaNs (Sodium Azide) - 0.1%

According to the weight of the lens prepare 10% homogenate. Reagents preparation: The tissue samples were used for the estimation of all parameters. The kidney were weighed and homogenate prepared by using 0.IN NaoH.

Protein estimation by lowry method:[7]

Reagents preparation:

Tris buffer (0.25M) solution:

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

Solution A:

lg of sodium carbonate and 200 mg of sodium hydroxide were weighed, transferred to a volumetric flask and the volume was made upto 50 ml of distilled water.

Solution B: 100mg of copper sulphate and 200mg of sodium potassium tartarate were weighed, transferred to a volumetric flask and the volume was made up to 10 ml with distilled water,

Alkaline copper solution:

50ml of solution A and Iml of solution B were transferred to a volumetric flask.

Folin ciocalteau phenol reagent:

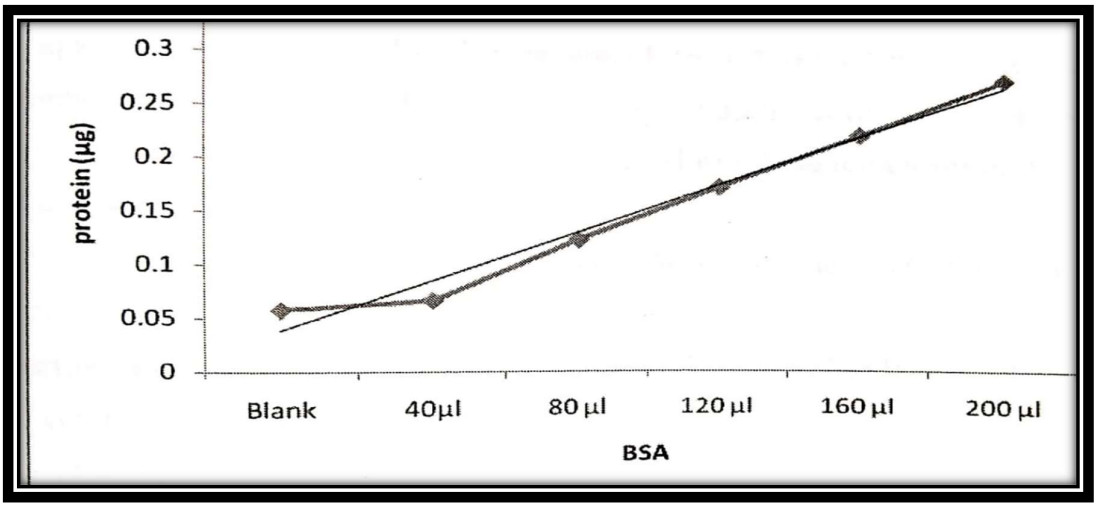
Folin ciocalteau phenol solution and distilled water were mixed in 1:1 ratio in a volumetric flask. Bovine serum albumin (0.1%) solution: 10mg of bovine serum albumin was weighed, transferred to a volumetric flask and made up to 10 with distilled water.

Method: (Lowry DH *et al*., 1951)

- Protein was estimated by the method developed by Lowry *et al* 1955.
- The sample volumes are based on tissue and diluted upto 400ul with buffer and add2ml of alkaline copper solution incubate for 10 minutes, add 0.2 ml of folin ciocalteau reagent and incubate for 45 minutes.
- Finally add 0.9 ml of distilled water. Measure the absorbance at S40n m by spectrophotometer.
- Protein levels were determined by comparing the known concentration of standardbovine serum albumin. The protein levels were expressed as mg protein/gm tissue.

Table 2: Standard graph BSA

BSA concentration	Protein estimation (µg)			
	Set 1	Set 2	mean	Mean after blanking
blank	0.060	0.056	0.058	-----
40µl	0.125	0.128	0.068	0.068
80 µl	0.186	0.187	1.28	0.064
120 µl	0.245	0.234	1.181	0.060
160 µl	0.299	0.284	0.233	0.058
200 µl	0.342	0.349	0.287	0.057



Estimation of protein carbonyl content:[8]

Procurement of chemicals:

Dintro phenyl hydrdrazine (DNPH), Trichloroacetic acid (TCA), EDTA, urea, from SRL.

1) 2N HCl: measure 10 ml of HCl and make up the volume with distilled water up to 52 ml.

2) 0.1% 2, 4 DNPH: Dissolve Img/ml in 2N HCl.

3) 20%Trichloro acetic acid: Weight 20gm oftrichloro acetic acid and make up the volume with distilled water up to 100ml.

4) 1.3M Tris EDTA pH7.4: Weight 5.74gm of tris HCl and 4.84 gm of EDTA dissolved in distilled water make up the volume up to 100 ml and adjust the pH to 7.4.

5) Urea: Weight 48gm of urea dissolved in 100ml of distilled water,

Procedure:

500ul of samples diluted with 500 ul of distilled water. To this add 500 ul of 2,4 DNPH. Incubate this mixture for l hour at room temperature then add 700 of ice cold TCA. Centrifuge at 10,000xg for 20 minutes. Discard the supernatant and separate the precipitate. Wash this pellet3 times with Iml of 1:1 ratio of ethanol: ethyl acetate. Finally dissolve the pellet with 0.9ml of urea, 0. Iml of Tris EDTA. Measure the absorbance at 365n m.

Estimation of superoxide dismutase: [9]

1) Tris buffer (0.256 M, Ph-8.3) solution:

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

2) Sodium Pyrophosphate Buffer (0.052M) solution:

1.38 g of Sodium Pyrophosphate was weighed, transferred to a volumetric flask the volume made up to 100ml with distilled water. PH of the solution is adjusted to 8.3 by using 0.025M HCI.

3) Phenazonium Methosulphate (PMS) (186 uM) solution:

5.69 mg Phenazonium Methosulphate was weighed and transferred to volumetric flask and the volume made up to looml distilled water.

4) Nitroblue Tetrazolium (NBT) (300 uM) Solution:

12.25 mg Nitroblue Tetrazolium was weighed and transferred to a volumetric flask and the volume made up to 50ml distilled water.

5) Nicotinamide adenine dinuclotide (reduced form) (NADH) (780uM) Solution:

13.83 mg NADH was weighed and transferred to volumetric flask and the Volume made up to 25 ml with distilled water.

Procedure for tissue SOD:[10]

Superoxide dismutase (SOD) activity was determined spectrophotometrically by the method developed by Kakkar *et al*. 1984.

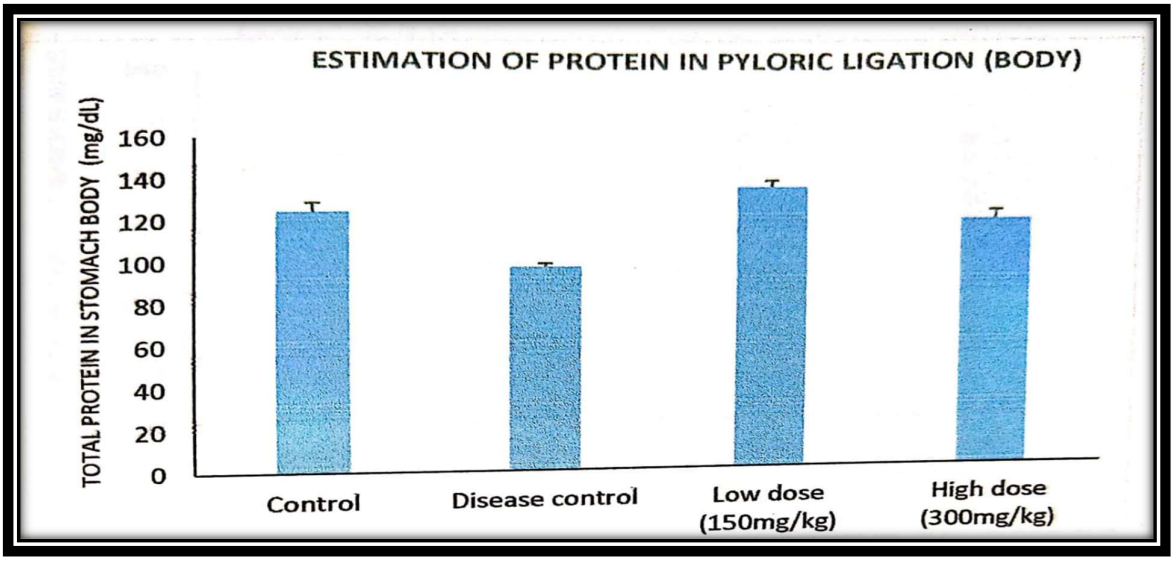
- To 0.1 ml of 10% (w/v) tissue homogenate prepared l in 0.25M Tris buffer under cold conditions.
- The homogenate was centrifuged at 10,000 rpm for 1Smin at 4°C.
- Aliquot of supernatant 0.1ml was added to 1.2 ml of 0.052 M sodium Dyrophosphate buffer (pH 8.3) followed by the addition of 0.1 ml of 186ul phenazoniummethosulphate, 0.3 ml of 300uM nitrobluetetrazolium, 0.2 ml of780uM NADH.
- Reaction mixture was incubated for 90 sec at 30° C, and the reaction was stopped by the addition of 1.0 ml of glacial acetic acid.
- Reaction mixture was stirred vigorously and shaken with 4.0 ml of n-butanol and centrifuged at 4000 rpm for 10 min.
- The absorbance of organic layer was measured at 560nm. A control was prepared using 0.I ml of distilled water devoid 0.I ml of homogenate.
- One unit of the enzyme activity is defined as the amount of enzyme that gaveS0% inhibition of NBT reduction in one minute. The SOD level was expressed.

RESULTS

Table 3: Effect of AQEA of total proteinin pyloric lygation induced ulcers in male albino wistar rats. in albino wistar rats

Body	Estimation of protein in pyloric ligation (µ moles of H_2O_2 Metabolized /mg of protein.						Mean ± SEM
group	R1	R2	R3	R4	R5	R6	
control	125.2	114.7	136.3	109.5	127.7	134.1	124.5±4.33
Disease control	97.3	103.4	95.5	100.2	98.2	92.3	97.81±1.56#
AQEA (150mg/kg)	141.8	139.3	129.5	132.7	145.4	125.7	135.7±3.11***
AQEA (300mg/kg)	108.8	126.9	114.7	111.7	126.4	133.9	120.4 ± 4.09***

All values represented MEAN±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}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

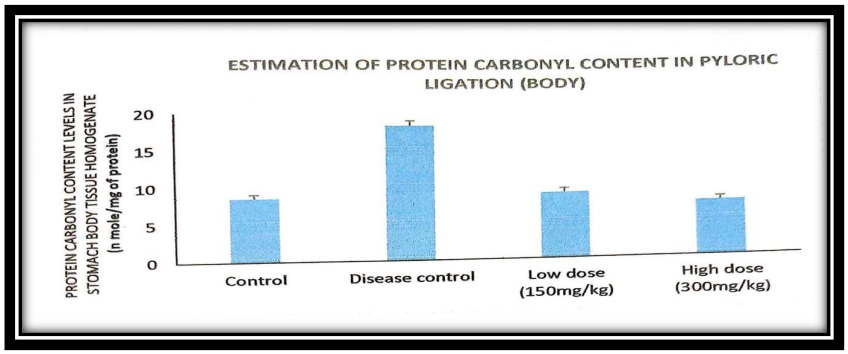


Graph 1: Effect of AQEA oftotal protein in pyloric lygation induced ulcers in male albino wistar rats. In albino wistar rats

Table 4: Effect of AQEA of total protein carbonyl content in pyloric lygation induced ulcers in male albino wistar rats. in albino wistar rats

Body	Estimation of protein carbonyl content in pyloric ligation (µ moles of H_2O_2 Metabolized /mg of protein.						Mean ± SEM
group	R1	R2	R3	R4	R5	R6	
control	8.1	4.7	73	9.9	8.3	6.2	7.41±0.7
Disease control	11.8	12.2	13.6	10.9	12.5	14.2	12.53±0.4#
AQEA (150mg/kg)	8.9	9.1	9.6	8.5	7.9	6.9	8.48±0.3***
AQEA (300mg/kg)	7.8	7.3	6.8	7.8	6.4	6.7	7.13 ± 0.2***

All values represented MEAN±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and ^{ns}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

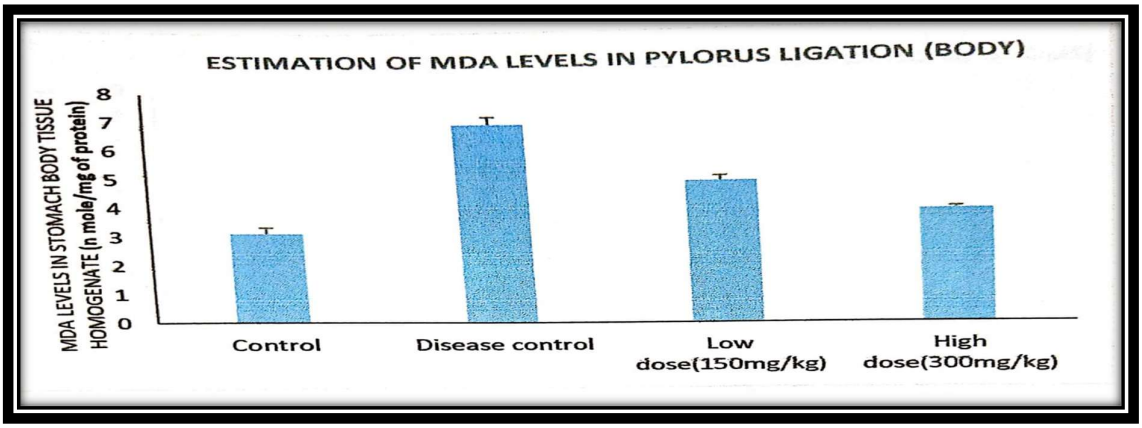


Graph 2: Effect of AQEA oftotal protein carbonyl content in pyloric lygation induced ulcers in male albino wistar rats. In albino wistar rats

Table 5: effect of AQEA of SOD in pyloric lygation induced ulcers in male albino wistar rats. in albino wistar rats

Body	Estimation of SOD in pyloric ligation (µ moles of H_2O_2 Metabolized /mg of protein.						Mean ± SEM
group	R1	R2	R3	R4	R5	R6	
control	8.5	4.78	73	9.98	8.3	6.2	7.41±0.7
Disease control	13.7	12.29	13.68	10.91	12.5	14.2	12.58±0.4#
AQEA (150mg/kg)	8.99	9.11	9.69	8.56	7.90	6.97	8.49±0.3***
AQEA (300mg/kg)	7.808	7.3	6.80	7.80	6.45	6.78	7.63 ± 0.2***

All values represented MEAN±SEM, n=6, ***P<0.001, **P<0.01, *P<0.05 and nsP<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 ofSOD in pyloric lygation 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 haemopoeitic 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. (Daya L

et al., 2012). Hence, effortsare 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. 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 poly phenolic 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-steroidal 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 HC1 in excess amounts from the parietal cells and auto digestion of mucosa by the gastric juice. In this study, catalase, estimation of protein content, estimation of protein carbonyl content, estimation of SOD 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+ 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 anti-secretory, antacid, antioxidant and anti-inflammatory activities. The pharmacological actions of Equisetum arvense due to the presence of chemical constituents.

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