International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS)

Research Article

ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACT FROM STEMS OF *IPOMOEA PES-CAPRAE (L.) R.Br* IN WISTAR ALBINO RATS

*[1]N.Deepak Venkataraman, [2]W.Clement Atlee and [3]T.PurushothPrabhu
[1]Dept. of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai-600097.
[3]Asst. Professor, Dept. of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai-600097.
[3]Asst. Professor, Dept. of Pharmacognosy, C.L.Baid Metha College of Pharmacy, Chennai-600097.

Corresponding Author: N.Deepak Venkataraman, **Email:** deepakvenkataraman.n@gmail.com

Abstract: *Ipomoea Pes-caprae(L.) R.Br* (IP)distributed in the tropics and subtropics regions is used in folk and tribal medicines. Traditionally IP was used to treat arthritis, pain, ulcer, cancer and wounds. The present study aims to discover the anti-ulcer effect of ethanolic extract from stems of IP (EESIP) at doses 200 mg/kg & 400 mg/kg in aspirin and pylorus ligature induced gastric ulcer models in terms ofprotection from lipid peroxidation and the antiulcer activity as seen by the area of gastric lesions, gastric juice volume, gastric pH, total acidity and total adherent gastric mucus content. Ranitidine (27 mg/kg) was used as the standard. Preliminary phytochemical study of EESIP revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols and terpenoids. The LD_{50} ofEESIPwas found to be >2000 mg/kg by acute oral toxicity study. EESIP produced dose dependent and significant anti-ulcer activity when compared to control group animals. Presence of flavonoids, tannins, sterols and terpenoids may be responsible for the anti-ulcer activity of EESIP.

Keywords: *Ipomoea Pes-caprae(L.) R.Br*, preliminary phytochemical study, anti-ulcer activity

INTRODUCTION:

A localized loss of gastric and duodenal mucosa leads to the formation of gastric and duodenal ulcers (together known as peptic ulcer) respectively. Peptic ulcer arises when the normal mucosal defensive factors (mucus, mucosal blood flow, formation of HCO_3 and PGE_2) are impaired by the aggressive factors (acid, pepsin, NSAIDs and *Helicobacter pylori*)[1].

Anti-ulcer drugs produce side effects like laxative effect or constipation (antacids), glaucoma, urinary retention, rapid heartbeat and mouth dryness (anticholinergics), head ache, dizziness, diarrhea and muscular pain (H_2 antagonists)[2]. Because of the above cited demerits, plant remedies are sought after as they are free of side effects, cost effective and act through multiple mechanisms.*Ipomoea Pes-caprae (L.) R.Br* is medicinally a valuable plant which was traditionally used to treat arthritis,pain, ulcer, cancer and wounds[3]. Thepresent study demonstrates the anti-ulcer potential of EESIP in aspirin and pylorus ligature induced gastric ulcer models.

MATERIALS AND METHODS:
Plant Material:

Whole plant of IP was collected from coastal areas ofKanyakumari district, Tamil Nadu and authenticated by Dr.P.Jayaraman (Botanist), Director PARC, West Tambaram, Chennai. The stems were segregated, shade dried, powdered and stored in air tight containers for future use.

Preparation of Ethanolic extract of stems of *Ipomoea pes-caprae*:

1 kg of dried stem was coarsely powdered and it was sieved using sieve number 60. Extraction process was carried out using 70% ethanol for 8 hours at temperature of 40°C bysoxlet apparatus after air drying the coarse powder. The extract thus obtained was allowed to stand at room temperature for 24 hrs. A semi-solid mass was obtained after it was filtered and concentrated by rotary vacuum pump. The percentage yieldwas found to be 2.41%.

Preliminary phytochemical screening:

The ethanolic extracts were subjected to phytochemical chemical tests to identify the phytoconstituents using standard qualitative reagents[4,5].

Animals used:

Adult male Wistar albino rats (180-250 g) were used. They were housed in standard animal cages in the Animal House section of the Department of Pharmacology, C.L.BaidMetha College of Pharmacy. They were given standard laboratory animal diet and water *ad libitum.*The animal studies were carried out with the institutional animal ethical committee clearance (Ref:IAEC/I/02/CLBMCP/2012 dated 28.08.2012).

Acute oral toxicity (OECD 423):

Acute toxicity study was carried out as per OECD guideline 423. Since available information suggests that mortality is unlikely at the highest starting dose level of 2000 mg/kg body weight, a limit test was conducted

straightaway at the dose of 2000 mg/kg. Animals were divided into 2 groups of 3 animals each.
Group I- treated with 1 % CMC P.O.
Group II- treated with 2000 mg/kg EESIP P.O.

The animals were observed for 14 days for signs of toxicity and changes in body weight after administration of single dose of EESIP 2000 mg/kg.

Aspirin-induced gastric ulcer:
Animals were divided into 4 groups containing 6 animals each. Group I received 2 ml/kg vehicle (1% gum acacia), group II received ranitidine (27 mg/kg body weight), groups III and IV received EESIP 200 mg/kg and EESIP 400 mg/kg respectively, *per orally*. The animals were then fasted (with free access to water) for a period of 24 h so as to ensure complete gastric emptying and a steady state gastric acid secretion. The 24 h fasted animals were again administered with the drugs/vehicle on the morning of the experiment. Sixty minutes after administration of the drugs/vehicle, aspirin was administered in a dose of 500 mg/kg body weight orally to all the animals[6]. Food was withheld for a duration of 5 more hours. Animals were then sacrificed by an overdose of anesthetic ether. The stomach was dissected out and a small opening was made along the greater curvature. All the gastric content was drained into a graduated centrifuge tube and used for biochemical estimations. The stomach was then cut open along the greater curvature and evenly spread out on a dissection board. A transparent film was placed over it and the boundary of the stomach and ulcerated area was traced on the film. The mucosal surface was then gently scraped with a blunt surface to collect the adherent mucus.

Pyloric ligature induced gastric ulcers:
Animals were divided into 4 groups containing 6 each and drugs/vehicle was administered as mentioned under aspirin induced gastric ulcers. The animals were then fasted (with free access to water) for a period of 24 h so as to ensure complete gastric emptying and a steady state gastric acid secretion. The 24 h fasted animals were again administered with the drugs/vehicle on the morning of the experiment. Sixty minutes after administration of the drugs/vehicle, the animals were anaesthetized using anesthetic ether and a midline incision was made just below the xiphoid process. The stomach was lifted out and ligated at the level of the pylorus following which it was replaced and the abdomen wall was closed by interrupted sutures[7]. The animals were then housed separately and food and water was withheld for a duration of 4 h following which they were sacrificed by an overdose of anesthetic ether. The stomach was then dissected out, gastric contents were collected and the boundary and ulcerated area was traced as mentioned above.

Determination of ulcer index:
The tracing of the stomach boundary and the ulcerated area on the transparent film was placed on top of a graph paper. The total surface area of the stomach and the lesions was determined in mm^2 from the graph paper. The ratio of total surface area and the total ulcerated area was determined and scoring of the ulcer index was done

according to the method described by Ganguly[8]. Percentage protection was calculated in the drug treated groups against control using the formula:

% protection = (1-ulcer index in test/ulcer index in control) x 100

Estimation of gastric volume, pH, and total acidity:
The gastric content that was transferred into centrifuge tubes was used for estimation of gastric volume, pH and total acidity. The tubes were centrifuged at 1000 rpm for 10 min and the gastric volume was directly read from the graduation on the tubes. The supernatant was then collected and pH was determined by using a digital pH meter (ECOSCAN: EC-PH510, Thermo Fisher Scientific, Mumbai). Total acidity was determined by titrating 1.0 ml of gastric juice against N/10 NaOH to pH 7 using phenolphthalein as the indicator and was expressed in terms of clinical units, *i.e.*, the amount (ml) of N/10 NaOH required to titrate 100 ml of gastric secretion[9].

Determination of adherent gastric mucus:
The mucosal scrapings were weighed and incubated with 1 per cent alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The tubes were then centrifuged at 1450 g for 10 min and the absorbance of the supernatant was measured at 489 nm to determine the total adherent mucopolysaccharide content[10].

Estimation of tissue malondialdehyde:
The stomach of the animals was weighed, homogenized (10% in cold 2 mM phosphate buffer, pH-7.2) and centrifuged to pellet out organic debris. The supernatant was then collected and malondialdehyde (MDA) was estimated in it as thiobarbituric acid reactive substances (TBARS)[11].

Statistical analysis:
Results were expressed as Mean ± Standard error. The statistical analyses were carried out by one way anova followed by Dunnett's multiple comparison test using Graph pad prism. P values less than 0.05 were considered significant.

RESULTS:
Preliminary phytochemical study:
Preliminary phytochemical study of EESIP revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols and terpenoids.

Acute oral toxicity study (OECD GL 423):
No signs of toxicity and no body weight changes were observed during the 14 days observation. The LD_{50} of EESIP was found to be >2000 mg/kg by acute oral toxicity study.

Aspirin induced gastric ulcers in rats:
EESIP showed a dose dependent protection against aspirin induced ulcers in rats (Table 1). Maximum protection was seen in the Ranitidine treated group. EESIP 400 mg/kg treatment produced significant (P<0.05) reduction of ulcer index. EESIP400 mg/kg and Ranitidine

exhibitedsignificant (P<0.05) reduction in lipid peroxidation products in the stomach tissue. There was a significant (P<0.05) reduction in volume of gastric secretion and total acidity in all drug treated groups as compared to control with ranitidine showing the maximum activity except Group III.Gastric pH was also found to be significantly (P<0.05) increased in all drug treated groups as compared to control, with maximum increase being produced by Ranitidine except Group III. Adherent gastric mucus content was also significantly(P<0.05) increased in all the drug treated groups as compared to control with ranitidine showing the maximum increase except Group III(Table I). The effect of 200 mg/kg of EESIP was non-significant in all parameters tested.

Pyloric ligature induced gastric ulcers in rats:
The extent of gastric ulceration in the control group was more severe in the pyloric ligature model (Table 2) as compared to the aspirin induced gastric ulcer model. Ranitidine (27/mg/kg) and EESIP (400 mg/kg) treatments produced a significant (P<0.05) reduction in the ulcer index with Ranitidine being superior. The percentage protection of Ranitidine was higher when compared with EESIP treated groups. EESIP (400 mg/kg) and Ranitidine treatments showed a significant (P<0.05) reduction in lipid peroxidation products, gastric secretion and total acidity compared to control.EESIP produced a dose dependent reduction of lipid peroxidation products, gastric ulcer index, juice volume and total acidity. Gastric pH was also found to be significantly (P<0.05) increased in Groups II & IV as compared to control with maximum increase being produced by Ranitidine. Adherent gastric mucus content was also found to be significantly(P<0.05) increased in EESIP 400 mg/kg and Ranitidine treated groups. But EESIP 200 mg/kg treatment did not have any significant effect on all the parameters evaluated (Table 2).

Table 1: Potency of EESIPin aspirin induced gastric ulcers in rats

Group	Treatment	Ulcer index (% protection)	MDA (nM/g tissue)	Gastric volume (ml/100 g body weight)	Gastric pH	Total acidity (clinical units)	Adherent gastric mucus (mg/g tissue)
I	Control	0.67 ± 0.06	111.09 ± 0.54	5.26 ± 0.09	3.33 ± 0.05	96.23 ± 2.59	143.31 ± 3.69
II	Ranitidine (27 mg/kg)	0.44 ± 0.08** (34.33)	73.83 ± 0.34**	3.29 ± 0.01**	4.72 ± 0.07**	61.96 ± 1.32**	166.09 ± 4.04**
III	EESIP (200 mg/kg)	0.58 ± 0.09 (13.43)	99.96 ± 0.57	4.99 ± 0.88	3.80 ± 0.25	91.28 ± 1.26	146.63 ± 1.21
IV	EESIP (400 mg/kg)	0.53 ± 0.05* (20.90)	88.78 ± 0.28*	4.55 ± 0.08*	4.20 ± 0.05*	81.73 ± 1.73*	153.03 ± 3.12*

Data expressed as mean ± SEM (n=6). Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison, P*<0.05 as compared to control

Table 2: Potency of EESIPin pyloric ligature induced gastric ulcers in rats

Group	Treatment	Ulcer index (% protection)	MDA (nM/g tissue)	Gastric volume (ml/100 g body weight)	Gastric pH	Total acidity (clinical units)	Adherent gastric mucus (mg/g tissue)
I	Control	0.72 ± 0.03	151.15 ± 0.46	5.69 ± 0.21	1.78 ± 0.05	121.79 ± 2.76	194.98 ± 5.27
II	Ranitidine (27 mg/kg)	0.43 ± 0.06** (40.28)	105.62 ± 0.29**	3.85 ± 0.050**	3.96 ± 0.23**	73.50 ± 1.34**	223.30 ± 2.06**
III	EESIP (200 mg/kg)	0.65 ± 0.13 (9.72)	144.37 ± 0.79	5.11 ± 0.94	3.29 ± 0.91	117.73 ± 2.35	202.81 ± 4.80
IV	EESIP (400 mg/kg)	0.57 ± 0.07* (20.83)	129.57 ± 0.45*	4.81 ± 0.25*	2.85 ± 0.42*	109.52 ± 2.05*	212.92 ± 1.68*

Data expressed as mean ± SEM (n=6). Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison, P*<0.05 as compared to control

DISCUSSION:
The results of the study reveal the significant and dose dependent anti-ulcer activity of ethanolic extract of stems from IP in aspirin and pylorus ligature induced gastric ulcer models. Only higher dose (400 mg/kg) of EESIP was significant but lower dose of 200 mg/kg was non-

significant.Aspirin induced ulcers develop due to the decrease in mucous production and increased proton back diffusion[12]. On the other hand, pyloric ligature induced ulcers develop as a result of accumulation of the gastric acid and distention of the stomach which in turn weakens the mucosal defenses[14].Estimation of gastric mucus which is known to possess cytoprotective effect (defensive factor) is more important inaspirin induced ulcer model as the secretion of this adherent mucus is dependent on prostacyclin synthesis which is inhibited by aspirin like drugs[13].

Ranitidinewas more effective in pylorus ligature induced ulcer model because gastric acid being the main causative factor of ulcer in this case. The reduction in total acidity and gastric volume by EESIP indicates the presence of constituents possessing anti-secretory effects. The increase in gastric mucus can be attributed to the presence of gastroprotective constituents in the extract. The extract may also contain antioxidants responsible for reduction in lipid peroxidation products.

Preliminary phytochemical screening exposed the presence ofalkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols and terpenoids. Flavonoids are known to have anti-secretory, cytoprotective and antioxidant activities[14]. Tannins act as astringents, precipitating microproteins on the ulcer site, thereby forming an impervious layer over the lining that hinders induced gastric ulcer in rats as evidenced by the gut secretions and protects the underlying mucosa(gastroprotective effect).[15-17]Sterols and terpenoids also have free radical scavenging activities.[18,19]

CONCLUSION:
Phytoconstituents such as flavoniods, tannins, sterols and terpenoids together with other constituents may be responsible for the significant anti-ulcer effect of EESIP in aspirin and pyloric ligature induced ulcer models. Further studies are required to ascertain the exact anti-ulcer mechanism and the constituents responsible.

REFERENCES:
1. HL Sharma and KK Sharma. Principles of Pharmacology, First Edition,Paras Publishing, New Delhi,**2008**; 386-399.
2. Richard A. Harvey and Pamela C. Champe, Lippincott's Illustrated Reviews: Pharmacology, Forth Edition, Lippincott Williams & Wilkins publishing, New Delhi, **2009**; 329-339.
3. Kirtikar Basu. A text book of Indian Medicinal Plants, Vol. III,Second Edition,Lalit Mohan Basu Publication, Allahabad, **2006**; 1726.
4. Kokate CK. Practical Pharma cognosy, Fourth Edition,Vallabh Prakashan, Delhi,**2005**;20-30.
5. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, Sixteenth Edition, Nirali Prakashan, Pune, **2006**;149-56.
6. Vinod Nair, Albina Arjuman, H.N. Gopalakrishna, P. Dorababu, Mirshad P.V, Divya Bhargavan, Dipsanker Chatterji.Evaluation of the anti-ulcer activity of NR-ANX-C (a polyherbal formulation) in aspirin & pyloric ligature induced gastric ulcers in albino rats, *Indian J. Med. Res.*, **2010**;13(2): 218-223.
7. Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M and Siplet H. A simple method for the uniform production of gastric ulceration in the rat,*Gastroenterology*, **1945**;5: 43-61.
8. Shah Chandragopal, Shiv Kumar, Battiwala Archana. Evaluation of Anti-ulcer activity of *Rosa Centifolia* (Linn.) flowers in experimental rats,*J. Nat. Rem*, **2012**;12(1): 20-29.
9. SS Sakat, Preeti Tupe, Archana Juvekar. Gastroprotective effect of *Oxalis corniculata* (whole plant) on experimentally induced gastric ulceration in Wistar rats. *Indian J. Pharm. Sci.*, **2012**; 74(1):48-53.
10. Anderson Luiz-Ferreira, Maira Cola, Victor Barbastefano, Felipe Meira de-Faria, Ana Beatriz A. de Almeida, Elisângela Farias-Silva, Tamara Regina Calvo, Clélia A. Hiruma-Lima, Wagner Vilegas, Alba Regina M. Souza-Brito. Healing Antioxidant and Cytoprotective Properties of *Indigoferatruxillensis* in Different Models of Gastric Ulcer in Rats, *Int. J. Mol. Sci.*, **2012**;13: 14973-14991.
11. Dillioglugil MO, Mekık H, Muezzinoglu B, Ozkan TA, Demir CG, Dillioglugil O. Blood and tissue nitric oxide and malondialdehyde are prognostic indicators of localized prostate cancer, *Int. Urol.Nephrol.*, **2012;**44(6),1691-6.
12. E. Tamil Jothi, G.Vimala Devi, Ch. Vamsi Anil Krishna, V.Suba. Int. J. Res.Analgesic, anti-inflammatory and anti-ulcer activity of Ethanol and ethyl acetate extracts of Tecomaria Capenis Leaves, *Ayurveda Pharm.*, **2013**; 4(3), 430-436.
13. Sanyal AK, Mitra PK,Goel RK. A modified method to estimate dissolved mucosubstances in gastric juice. *Indian J. Exp. Biol.*,**1983**;21: 78-80.
14. Kelly Samara de Lira Mota, Guilherme Eduardo Nunes Dias, Meri Emili Ferreira Pinto, ÂndersonLuiz-Ferreira, Alba Regina Monteiro Souza-Brito, Clélia Akiko Hiruma-Lima, José Maria Barbosa-Filho,Leônia Maria Batista. Flavonoids with GastroprotectiveActivity. *Molecules*, **2009**; 14: 979-1012.
15. Nwafor, P.A, K.D. Effrain, T.W. Jack. Gastroprotective effects of aqueous extract of Khayasenegalensis bark on indonethacin-induced ulceration in rats. *West African. J. Pharmacol.Drug Res*,**1996**; 12: 46-50.
16. Nwafor P.A, F.K. Okwuasaba, L.G. Binda. Antidiarrheal and anti-ulcerogenic effects of methanolic extract of Asparagus pubescens root in rats. *J.Ethanopharmacol.*,**2000**; 72: 421-427.
17. Al-Rehailey A.J, T.A. Al-Howiriny, M.O. Al Sohaibani, S. Rafatullah. Gastroprotective effects of 'Amla' Emblicaofficinalison in vivo test models in rats. *Phytomedicine*, **2002**; 9: 515-522.
18. Lucía Plaza, Concepción Sánchez-Moreno, Sonia de Pascual-Teresa, Begoña de Ancos, M. Pilar Cano. Fatty Acids, Sterols, and Antioxidant Activity in Minimally Processed Avocados during

Refrigerated Storage. *J. Agric. Food Chem*, **2009**;57 (8): 3204-3209.

19. Grassmann J. Terpenoids as plant antioxidants. *Vitam.Horm.*, **2005**; 72: 505-35.