

PHARMACOLOGICAL SCREENING AND EVALUATION OF ANTI-PEPTIC ULCER PROPERTY OF LEAVES OF ARTOCARPUS INTEGRIFOLIA

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Received: 08 Dec 2013, Revised and Accepted: 06 Feb 2014

ABSTRACT

Objective: Pharmacological screening and evaluation of anti-peptic ulcer property of leaves of *Artocarpus integrifolia*.

Methods: The methanolic extract of *Artocarpus integrifolia* was used for evaluation of anti-peptic ulcer activity by employing pylorus ligation induced ulcer model in rats at 250 mg/kg body weight p.o.

Results: The extract 250 mg/kg showed significant ($P < 0.01$) reduction in gastric volume, total acidity, increase in pH, ($P < 0.05$) and reduced free acidity as compared to control group. Further it was observed that ulcer index of extract treated was also reduced significantly ($P < 0.01$) as compared to control group and showed 50.90% ulcer protection.

Conclusion: This present study indicates that methanolic extract of *Artocarpus integrifolia* leaves has significant anti-peptic ulcer activity.

Keywords: *Artocarpus integrifolia*, Pylorus ligation, Anti-peptic ulcer activity, Omeprazole.

INTRODUCTION

In the present scenario, natural medicines are gaining prominence, because they are economical, easily available and relatively free from side effects. The use of plants extract and pure compounds isolated from natural sources provides foundation to modern pharmaceutical compounds. The well known Indian systems of medicine namely, Ayurveda, Siddha and Unani have used plant based raw material.[1].

There are 50 species of genus *Artocarpus* (Moraceae) of evergreen and deciduous trees. The genus is important source of edible fruit and is widely used in traditional medicines. *A. integrifolia* (Moraceae) is mentioned in Ayurveda and is reported to possess antibacterial, anti-inflammatory, antidiabetic, antioxidant and immunomodulatory properties.

A. integrifolia is reported to contain artocarpesin, norartocarpesins, betullic acid, cycloheterophyllin, triterpenic compounds like cycloartenyl acetate, cycloartenone, heterophyllol (phenolic), 3.3% tannin, 2, 4', 6'-- trioxxygenated flavanones, heteroflavanones A and B, β -sitosterol, phenolic compounds including flavonoids, jacinine, (lactone) and arylbenzofurans.[2].

Since 1960, ulcer-related physician visits, hospitalizations, operations, and deaths have declined in India by more than 50%, primarily because of decreased rates of PUD (peptic ulcer disease) among men. Although the overall mortality from PUD has decreased, death rates have increased in patients older than 75 years of age, most likely as a result of increased consumption of NSAIDs and an ageing population.[3]. Ulcer is a conglomerate of heterogeneous disorders, which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and/or pepsin [4]. WHO has encouraged studies for treatment and prevention of ulcer disease using traditional medical practices. The present study is aimed to evaluate the traditional claim of anti-peptic ulcer activity of plant *A. integrifolia*.

MATERIALS AND METHODS

This study was conducted in the pharmacology laboratory, department of pharmacology, Rajiv Academy for Pharmacy, Mathura, India.

Collection and extraction

The fresh leaves of *A. integrifolia* were collected from local area of Mathura, India in the month of August 2012. The material was taxonomically identified, confirmed and authenticated by Dr. Sunita

Garg (Chief Scientist and Head of Raw Material Herbarium and Museum), NISCAIR, New Delhi. A Voucher specimen NISCAIR/RHMD/ Consult/2013/2217/223 is deposited there.

The collected leaves were washed 2-3 times with tap water to remove adhering dust and allowed to dry in shade. The dried material was crushed to coarse powder with mechanical grinder. It was then passed through the 40 No. mesh sieve. The powder was stored in air tight container..

A weighed quantity (150 g) of the powder was subjected to continuous hot extraction in Soxhlet apparatus with methanol as a solvent and extracted till the solvent became colourless. Extract was evaporated under reduced pressure using rotary evaporator at a low temperature of 40-60°C until the extract turned syrupy and then this syrupy extract was transferred to an evaporating dish for drying on a water bath.

Animals

Healthy albino wistar rats weighing 200-250 g were used for the study. The animals were housed under standard environmental conditions (22±3°C and 35-60% relative humidity). Food was withdrawn 24 hours before the experiment, but animals were allowed free access of water.

The experimental protocol was approved by the Institutional animal ethical committee for animal experimentation of, Rajiv Academy for pharmacy, Mathura, India., with registration No.- IAEC/RAP/3962.

Preliminary phytochemical screening of extract

Qualitative chemical tests were conducted for methanolic extract of *A. integrifolia* to identify the various phytoconstituents employing standard screening test [5]. Methanolic extract of *A. integrifolia* gave positive test for flavonoids, tannins and phenols.

Chemicals and Reagents

All the drugs and chemicals were of analytical grade. Standard Omeprazole and Topfer's reagent were procured as gift sample, from Centron Research labs, New Delhi.

Experimental Design

Albino wistar rats were divided into 3 groups, each group containing 6 rats. Group-I received vehicle control (1% SCMC, sodium carboxymethyl cellulose 1ml/kg, p.o.). Group-II received standard drug (Omeprazole - 20mg/kg, p.o.). Group-III received methanolic extract of *A. integrifolia*, (MEAL) leaves (250mg/kg, p.o.).

Pylorus ligation model

Extract of *A. integrifolia* leaves was screened for antiulcer activity by pylorus ligation method [6] In this method albino rats were fasted in individual cages for 24 hours prior to pyloric ligation. *A. integrifolia* methanolic extract of leaves, standard drug and control vehicle was administered 45 minutes prior to pylorus ligation under light ether anaesthesia. The abdomen was cut open by midline incision process, pyloric portion of the stomach was slightly lifted out and ligated avoiding damage to its blood supply[7,8].

The stomach was replaced carefully and the abdominal wall closed by sutures. After 3 hours of pylorus ligation, rats were sacrificed by an over dose of anaesthetic ether. The stomach was isolated, the content of the stomach drained into tubes and centrifuged at 2000 rpm for 10 min.

The supernatant was collected and used for further study. The stomach was then cut open along the greater curvature and washed slowly under running tap water and inner surface was examined for ulceration by giving score number. The severity of the ulcer was scored microscopically by 10X magnification lens. Mean ulcer score for each animal was expressed as ulcer index[9-11].

Ulcer Index (U.I.): Score

No coloured stomach -	0
Red coloration-	0.5
Spot ulceration-	1
Hemorrhagic streak-	1.5
Ulcer -	2
Perforation -	3

Percentage inhibition

Percentage inhibition was calculated using the following formula..

Percentage protection= Control U.I. –Test U.I. × 100

Control U.I

Statistical analysis

The results were expressed as Mean ±SEM, (n=6). Statistical analyses were performed by using one way ANOVA followed by Dunnett's test multiple comparison, where P<0.001, P<0.01, P<0.05 was considered statistically significant. Data were analyzed through graph pad software.

RESULTS

Phytochemical screening

The preliminary phytochemical screening of methanolic extract of *A. Integrifolia* (MEAI) leaves

showed the presence of carbohydrates, flavonoids, tannins and phenols.

Antiulcer activity by pylorus ligation method

MEAI at 250 mg/kg body weight treated rats showed significant (P<0.01) reduction in ulcer index when compared with control. The percentage protection against ulcer by Omeprazole, MEAI were found to be 61% and 50.90% respectively. MEAI produced significant (P<0.01) reduction in the volume of gastric acid secretion and an increase in the pH of the gastric juice. In addition, total acidity and free acidity were also reduced significantly as (P<0.01, P<0.05) shown in Table 1 and 2.

Table 1: Effect of Omeprazole and methanolic extraction of *A. integrifolia* leaves on gastric volume, pH, free acidity and total acidity in pylorus ligated model

Groups	Gastric volume	Gastric pH	Total acidity	Free acidity
Control	6.3±0.32	2.4±0.33	102±1.8	79.50±1.04
Std. (Omeprazole 20 mg/kg)	2.1±0.28***	6.3±0.24***	29±0.91***	21.00±0.9**
MEAI 250 mg/kg	4.3±0.24**	2.7±0.10**	39±0.85**	33.75±0.85*

Results were expressed as mean ± SEM for 6 rats, Statistical comparison was performed by Graph pad software using ANOVA with Dunnett's test. *** P<0.001, **P<0.01, *P<0.05 were considered statistically significant when compared with the control group.

Table 2: Effect of omeprazole and methanolic extraction of *A. integrifolia* leaves on ulcer index and percentage of ulcer protection in pylorus ligated model

Groups	Ulcer index	% protection
Control	11±0.24	----
Std. Omeprazole 20mg/kg)	4.2±0.13**	61%
MEAI 250mg/kg	5.4±0.17**	50.90%

Results were expressed as mean ± SEM for 6 rats, Statistical comparison was performed by Graph pad software using ANOVA with Dunnett's test. *** P<0.001, **P<0.01, *P<0.05 were considered statistically significant when compared with the control group.

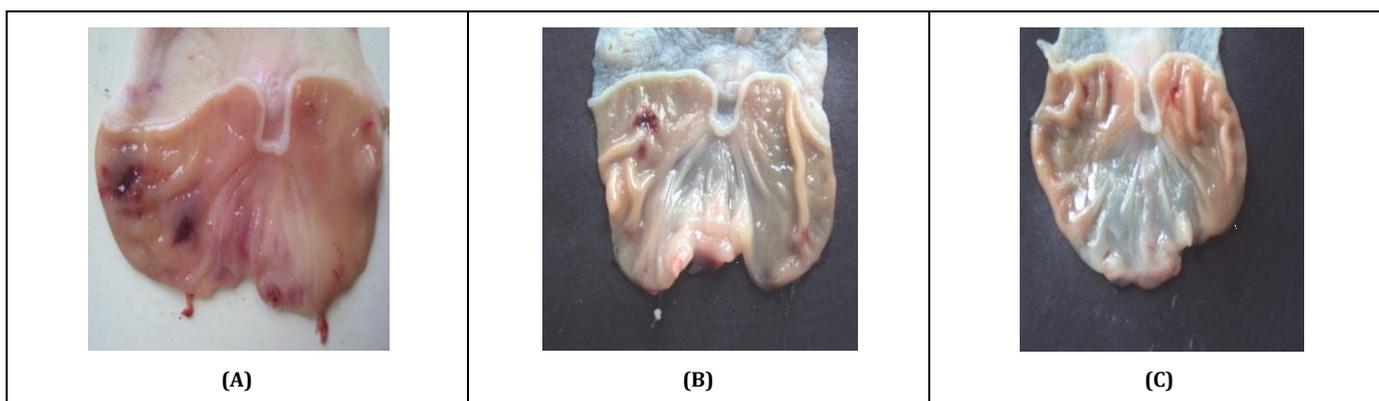


Fig. 1: Images of pylorus ligation induced ulcer model (A) Control, (B) Standard (Omeprazole- 20 mg/kg),and (C) MEAI – 250mg/kg.

DISCUSSION

Plants constitute a major remedy in the traditional medical system. The practice continues today due to their biomedical benefits, non toxic nature, their easy access and a place in cultural benefits. In the present scenario, medicinal plants have been used as a source of drugs and as a potential source of remedy for diseases that are currently difficult to treat/cure. Due to some reported side effects of available antiulcer drugs, focus has shifted towards natural products as the new sources of antiulcer agents.[12]

Review of the available literature revealed that majority of the antiulcer studies so far conducted have used pylorus ligation model [13]. The different constituents like flavonoids, tannins, phenols, steroids, saponins, alkaloids and glycosides have been reported to be responsible for anti ulcer activity [14].

Peptic ulcer describes a condition in which there is a discontinuity in the entire thickness of the gastric and duodenal mucosa that persists as a result of acid and pepsin in gastric juice. Peptic ulcer is caused by an imbalance between aggressive factors (e.g. acid, bacteria, NSAID's, dietary factors) and defensive factors (e.g. mucus, bicarbonate, prostaglandins).[15,16].

A. integrifolia extract is one such herbal drug used in the present study primarily to evaluate the anti ulcer activity by using pylorus ligation model in rats[17].

The causes of gastric ulcer in pyloric ligation is believed to be due to stress induced increase in gastric hydrochloric acid secretion. The volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid [18]. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. Pylorus ligation models are usually employed to observe the potential of anti ulcer drugs for their anti acid-secreting activity by checking the gastric volume and its effect on gastric pH, total acidity and free acidity.

The antiulcer property of *A. integrifolia* in pylorus ligation model is evident from its significant reduction in gastric volume, free acidity, total acidity and ulcer index and also, an increase in the pH, it is suggested that *A. integrifolia* has anti-secretory activity.

CONCLUSION

Anti ulcer activity of MEAI at dose 250 mg/kg decreased gastric acid volume and showed significant reduction in free acidity and total acidity in rats treated with it, and also indicate anti-secretory properties. From the results it can be concluded that the leaves of the plant of *A. integrifolia* posses gastro protective potential which may be due to the presence of tannins, flavonoids, and sterols present in it The present study indicates a significant decrease in ulcer index and increased percentage inhibition of ulceration.

ACKNOWLEDGEMENTS

The authors would like to thank Dr.Devender Pathak, director, and Dr.(Mrs.) Kamla Pathak, dean, and Head, Department of Pharmaceutics, Rajiv Academy for Pharmacy, Mathura for providing the facilities to carry out these investigations.

REFERENCES

1. Kar, A., 2007. Pharmacognosy and Pharmacobiotechnology, New Age International Private Limited. New Delhi, II edition, pp.5, 15.
2. Jagtap, U.B. and Bapat, V.A., 2010. *Artocarpus*, A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology, vol. 129, Iedition 2, pp. 142-166.
3. Dipiro, Joseph T., Talbert, Robert L., Yee, Gary C. and Wells, Barbara G., Pharmacotherapy:A Pathophysiologic Approach. Tata McGraw-hill Education Private Limited, New Delhi,2008, VII edition, pp. 569-572.
4. Tripathi, S., Pradhan, D. and Anjana, M., Anti- inflammatory and antiarthritic potential of *Ammania baccifera* Linn., International Journal of Pharma and Biosciences,2010, Vol. 1., pp. 1-7.
5. Kokate, C.K. and Purohit, A.P., Pharmacognosy. 38th edition., Nirali Prakshan, Pune, India, 2010, pp. 600-604. 83.
6. Shay, H., Konrav, S.A., Fels, S.S., Meraze, D., Gruenstein, M. and Siple, H., A simple method for uniform production of gastric ulceration in ras,Gastroenterology, 1945, Vol. 5, pp 43-61.
7. Deshpande, S.S., Shah, G.B. and Parmar, N.S. 2003. Antiulcer activity of *Tephrosia Purpurea* in rats., Indian Journal of Pharmacology, 1999, Vol. 35, pp. 168-172.,
8. Patil, B. P., Mahadik, J. V., Patil, B. S. and Naikwade, Evaluation of anti-ulcer activity of aqueous and ethanolic extract of *Oxalis Corniculata* leaf in experimental rats, International Journal of Pharmaceutical Research and Development, 2011, pp. 98-104.
9. Palanisamy, Kumar A. V., Permul P. and Billa, V., 2012. Anti-ulcer activity of ethanolic extract of *Annona Squamosa* Leaves. **International Journal of Pharmaceutical Research and Development**, pp. 162-167.
10. Patel, G., Nayak, S. and Shrivastava, S., 2010. Anti-ulcer activity of methanolic leaf extract of *Nerium Indicum*. International Journal of Biomedical Research, vol. 1, pp. 55-61.
11. Vogel, Wolfgang H., Scholkens, Bernward A., Sandow, J., Muller, G. and Vogel, Wolfgang F, Drug Discovery and Evaluation of Pharmacological Assays, II edition, Springer-Verlag, Berlin, 2002, pp. 867-870
12. Gregory M, Vithalrao KP, Franklin G, Kalaichelavan V. Anti-ulcer (ulcer-preventive) activity of *Ficus arnottiana* Miq. (Moraceae) leaf methanolic extract, Am.J. Pharmacol. Toxicol., 2009, 4(3):pp 89-93.
13. Dharmani, P. and Palit, G., Exploring Indian medicinal plants for antiulcer activity. Indian J Pharmacol, Vol. 38, I. 2, 2008, pp. 95-99.
14. Premila, M.S, A Clinical Guide to the Healing Plants of Traditional Indian Medicine. Haworth Press, New York,2006, pp. 20-32.
15. Babu, Dr. S. S, A treatise on home remedies, Pustak Mahal, New Delhi. 2005, pp 14-15.
16. Bajru, H.K,A Text book of Natural Home Remedies for common ailments. Orient paperbacks, New Delhi. 1995.pp 172-74.
17. Tripathi, K D., Essentials of Medical Pharmacology, Jaypee brothers medical publishers, New Delhi, VI edition, 2008, pp 628, 637.
18. Sakat SS, Juvekar RA, Antiulcer activity of ethanolic extract of leaves of *Erythrina indica*, Am. J. Pharmacol. Toxicol, 2009,, pp 96-100