Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

ANTIULCER ACTIVITY OF ANNONA RETICULATE LEAVES EXTRACT IN RATS

JAIDEEP SINGH[1]*, SANTHOSH KUMAR VR[2], VASUDHA KADAM[3]

[1] Sree Siddaganga college of Pharmacy, Tumkur, Karnataka 572102, [2]Al-Ameen college of Pharmacy, Bangalore, Karnataka, [3]Nagpur Veterinary College, Nagpur, Maharashtra. Email: jaydeepsingh21@gmail.com.

Received: 3 Sep 2011, Revised and Accepted: 2 Nov 2011

ABSTRACT

The present study has been undertaken to investigate the antiulcer activity of Annona reticulate leaves extract in rats. In Ethanol and indomethacine induced ulcer model, Extract or reference or vehicle was administered to 24 hours fasted rats, after 30 min, 50% alcohol or indomethacine was administered. After 1 hour, rats were sacrificed and ulcer index and related parameters were determined. Annona reticulate (100 & 200 mg/kg doses) and reference drug (3mg/kg) has shown significant decrease in ulcer index, acid volume and content and showed improvement in glutathione and pH when compared with vehicle control. Therefore, aqueous extract of leaves of Annona reticulate is a potent antiulcer agent.

Keywords: Antiulcer, Ethanol, Indomethacine, Ulcer index, Glutathione

INTRODUCTION

Peptic ulcer disease has become one of the most common gastrointestinal disorders that involves the damage to mucosal membrane or severe lesions.[1] Traditionally peptic ulcers have been described as an imbalance between the luminal acid peptic attack versus the mucosal defense.[2] Numerous plants have been evaluated as therapeutics for the treatment of a variety of diseases including peptic ulcer.[3,4] Annona reticulate (Annonaceae) is a tree commonly known as Ramphal. The leaf decoction is given as a vermifuge. Crushed leaves or a paste of the flesh may be poulticed on boils, abscesses and ulcers. The unripe fruit is rich in tannin is dried, pulverized and employed against diarrhea and dysentery. The bark is very astringent and the decoction is taken as a tonic and also as a remedy for diarrhea and dysentery. The root decoction is taken as a febrifuge.[5] The plant (mainly leaves) is rich of alkaloids, diterpenoids, acetogenins, δ-cadinene, elemol, α-copaene and δ-eudesmol in combination with monoterpenes terpinen-4-ol and α-terpineol.[6] Free radical scavenger property[7] and rich alkaloidal contents favours antiulcer activity of Annona reticulate.

The Annona reticulate leaves has been claimed to have antiulcer activity[5], but no detailed scientific investigations have been carried out to define the antiulcer activities of Annona reticulate. Thus the present investigation sets out to study the antiulcer activity of Annona reticulate leaves extract.

MATERIALS AND METHODS

Drugs

DTNB solution (5, 5-dithiobis-2-nitrobenzoic acid) was procured from M/S Sisco research laboratories Pvt. Ltd, Mumbai-400099, India.

Plant material

The leaves of Annona reticulate was collected from the outskirts of Tumkur. The plant was identified by a Botanist.

Animals

Wistar rats weighing 180-200 gm of either sex were used in this study. They were divided into four groups, with each group containing 6 animals. Clearance to carry out the work was obtained from the Institutional animal ethical committee bearing no. SSCPT/IAEC. Clear /51/2007–08 dated 22/09/2007

Preparation of extract

The AELAR (Aqueous extract of leaves of Annona reticulate) extract was prepared by soxhlet extraction of 100 g leaf powder in 250 ml of distilled water. The extract was concentrated dried in vacuum and residue stored in refrigerator at 2-8^{0}C for use in subsequent experiments.

Experimental design

In this study, there are four groups (n=6). Group-1 served as vehicle control and recieved indomethacine or ethanol. Group-2 served as reference and received famotidine (3mg/kg). Group-3 served as treated group and recieved AR (100mg/kg), Group-4 served as treated group and recieved AR (200mg/kg).

Ethanol-induced gastric lesions[8]

Annona reticulate or vehicle was administered to 24 hours fasted rats. After 30 min, ulceration was induced by oral administration of 50 % ethanol. The animals were sacrificed after 1 h following administration of ethanol. The stomach was removed, opened along the greater curvature and ulcer index was measured. The gastric contents were collected and centrifuged. Supernatant was collected and acid volume[9] and pH[10] were determined. Supernatant was titrated against 0.01 N NaOH to find out the total acidity.[11]

Indomethacin-induced gastric mucosal damage[12]

After fasting for 24 hours, Test or reference or vehicle has been administered. Indomethacin (10 mg/kg, p.o.) was administered by gavage needle after 30 min. of administration of test or vehicle. One hour after test or reference or vehicle administration, all rats were sacrificed. The numbers of ulcer spots in the glandular portion of the stomach were counted in both control and drug treated animals and the ulcer index was calculated.

Ulcer index[11,13]

Ulcer index was calculated by using the following score and formula:

Scoring of ulcer

0 = Normal coloured stomach, 0.5 = Red colouration, 1 = Spot ulcer, 1.5 = Haemorrhagic streaks, 2 = Ulcers ≥ 3 but ≤ 5, 3 = Ulcers >5

Calculation of ulcer Index

U1 = UN + US + UP x 10-1

U1 = Ulcer Index

UN = Average of number of ulcer per animal, US = Average of severity score,

UP = Percentage of animal with ulcer

GSH[14]

Glutathione was determine by Ellman's reaction using 5'5'-dithio-bis-2-nitrobenzoic acid (DTNB).

Statistical analyses

Data of independent observations are shown as the mean+sem. Statistical analysis was performed using unpaired t test. $P<0.05$ was considered as statistically significant.

RESULTS

In both the models, AELAR has showed significant dose dependent decrease in ulcer index (UI) when compared with vehicle control group (P<0.001). Reference drug also shown significant decrease in UI (P<0.001), (Table-1, 2). AELAR has showed significant decrease in acid volume and gastric content when compared with vehicle group (P<0.001), (Table-1, 2). However, AELAR and reference drug has showed significant increase in pH levels (P<0.001). Significant improvement in glutathione level has showed by AELAR which corroborate its free radical scavenging property (P<0.001), (Table-1, 2).

Table 1: Ethanol induced gastric ulcers

S. No.	Groups	Dose (mg/Kg)	UI	Acid volume	Total acidity	pH	Glutathione (mmol/mg protein)
1	Vehicle control	----	14.7±1.6	8.4±0.20	116.7±3.17	2.00± 0.09	4.4±0.30
2	Famotidine	3	3.8±1.5***	2.1±0.78***	64.23±2.48***	4.21±0.18***	7.3±0.45
3	AELAR	100	5.9±0.79***	4.1±0.24***	78.14±1.98***	3.11±0.19***	5.2±0.34
4	AELAR	200	4.2±1.02***	3.4±1.04***	65.69±3.14***	3.86 ±0.28***	8.7±0.22

n=6, Values represent the mean±sem. P<0.05*, P<0.001***.

Table 2: Indomethacine induced gastric ulcers

S. No.	Groups	Dose (mg/Kg)	UI	Acid volume	Total acidity	pH	Glutathione (mmol/mg protein)
1	Vehicle control	----	13.4±0.79	6.1±0.42	121.4±2.4	2.32±0.04	5.17±1.04
2	Famotidine	3	4.12±0.95***	2.8±0.64***	57.49±2.18***	3.47±0.18***	6.87±0.74***
3	AELAR	100	6.4±1.12***	4.2±1.10***	74.14±3.24***	3.07±1.02***	5.50±0.96**
4	AELAR	200	4.0±0.74***	3.7±0.75***	60.56±2.89***	3.36±0.09***	6.94±0.47***

n=6, Values represent the mean±sem. P<0.05*, P<0.001***.

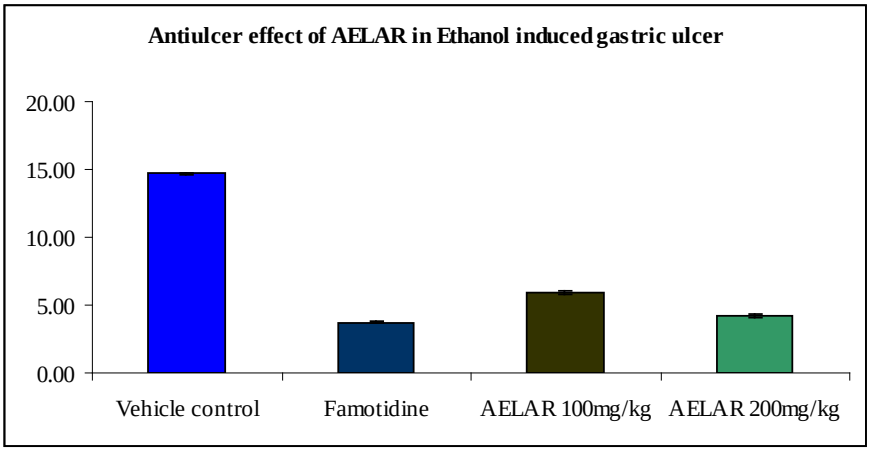

Fig. 1: Antiulcer effect of AELAR in Ethanol induced gastric ulcer

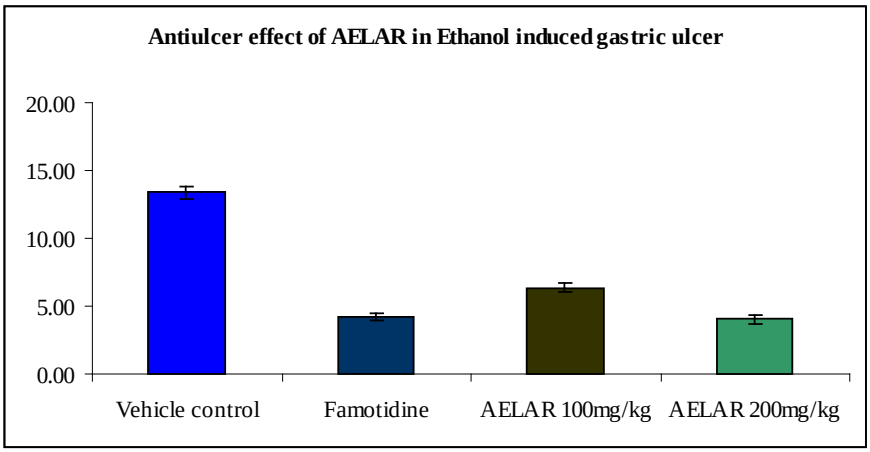

Fig. 2: Antiulcer effect of AELAR in Indomethacine induced gastric ulcer mucosal damage

DISCUSSION

Peptic ulcer is the most common gastrointestinal disorder which occur as a result of an imbalance between the aggressive factors of acid and pepsin and maintenance of mucosal integrity through endogenous defensive mechanism. To regain the balance, different therapeutics including spice and plant extracts have been used.[15]

Ulcer index is a index of ulcer formation and ulcer formation is directly related to factors such as gastric volume, total acidity, pH and also Glutathione. In both the model of ulcers, there is a increase in acid secretion, which in turn caused increase in gastric volume, low pH, increased total acidity resulting in higher ulcer index. AELAR reduced the gastric volume, total acidity and hence ulcer index showing the anti-secretary mechanism.[16] Indomethacin induced gastric mucosal injury decreased the glutathione peroxidase activity and aggravated the injury due to accelerated accumulation of H_2O_2 and lipid peroxidation.[17] Furthermore, excessive peroxidation causes increased glutathione consumption.[18] Peroxidation was also enhanced by ethanol. These effects were counteracted by enhancing the glutathione tissue level after treatment with AELAR which will serve as free radical scavenger.[19]

Ethanol induced gastric damage ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions, which is attributed mainly to the inhibition of biosynthesis of cytoprotective PG resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway.[20] These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions reducing the transmucosal potential difference and induce formation of erosions and ulcers. In this model AELAR was able to produce a significant reduction of the gastric mucosal damage, indicating a probable local increase in PG synthesis.[21]

Ulcer formation induced by Indomethacin, a non – steroidal anti – inflammatory agent, is known to be correlated with inhibition of cyclooxygenase, that prevents prostaglandin (PG) biosynthesis,[22,23] which in turn inhibits the release of mucus,[24] a defensive factor against gastrointestinal damage. In this model AELAR was produced its ulcer protective effect by counteracting the inhibition of PG synthesis and enhancing the mucus release.

In the present study, the anti ulcer effect might be due to a possible relationship between protection of mucosal damage, inhibition of acid secretion and the antioxidant property of Annona reticulate. Annona reticulate has already been reported for its antioxidant activity.[7] Therefore, the AELAR has showed its antiulcer activity due to its antisecretory, antioxidant and cytoprotective mechanism. This study indicates that AELAR has a potential anti ulcer activity.

REFERENCES

1. Falcao HS, Mariath IR, Diniz MF, Batista LM, Barbosa-Filho JM. Plants of the American continent with antiulcer activity. Phytomedicine 2008; 15: 132-146.
2. Bettarello A. Antiulcer therapy, Past to present. Digest Dis Sci 1985; 30: 36-42.
3. Devi RS, Kist M, Vani G, Devi CS. Effect of methanolic extract of Terminalia arjuna against Helicobacter pylori 26695 lipopolysaccharide-induced gastric ulcer in rats. J Pharm Pharmacol 2008; 60(4): 505-514.
4. Coelho RG, Gonzalez FG, Sannomiya M, Di Stasi LC, Vilegas W. Gastric anti-ulcer activity of leaf fractions obtained of polar extract from Wilbrandia ebracteata in mice. Nat Prod Res 2009; 23(1): 51- 59.
5. Morton J. Custard Apple. Miami (FL): In: Fruits of warm climates. 1987.
6. Ogunwande, Isiaka A, Ekundayo, Olusegun, Olawore, Nureni O, Kasali, Adeleke A. Essential Oil of Annona reticulata L. Leaves from Nigeria. Journal of Essential Oil Research 2006.
7. Baskar R, Rajeswari V, Sathish KT. Invitro antioxidant activity studies in leaves of Annona species. Indian Journal of Experimental biology 2007; 45: 480-485.
8. Robert A, Nezamis JE, Lancaster C, Hanchar A. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterology 1979; 77(03): 433-443.
9. Maity S, Vedasiromoni JR, Ganguly DK. Antiulcer effect of hot water extract of black tea (Camellia sinensis). J Ethnopharmacol 1986; 46: 167-174.
10. Rezq AA, Elmallh MM. Anti-ulcer effect of Cinnamon and Chamomile aqueous extracts in rat models. Journal of American Science 2010; 6(12): 209-216.
11. Kulkarni SK. Handbook of Experimental Pharmacology. 3rd ed. New Delhi (India): Vallabh Prakashan; 1999.
12. Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and duodenal antiulcer agents. Indian J Pharmacol 1993; 25: 120-135.
13. Gerhard Vogel H. Drug Discovery and Evaluation. New York (USA): Springer-Verlag Berlin Heidelberg; 2002.
14. Moron MA, Mannervick B. Levels of glutathione, glutathione s-transferase activities in rat liver. Biochemical et biophysica acta 1979; 582: 67-78.
15. Goel RK, Sairam K. Antiulcer drugs from indigenous sources with emphasis on Musa sapientum, tamrabhasma, Asparagus racemosus and Zingiber officinale. Indian J Pharmacol 2002; 34: 100–110.
16. Goel RK, Bhattacharya SK. Gastro duodenal mucosal defense and mucosal protective agents. Indian J Expl Biol 1991; 29: 701-14.
17. Yoshikawa T, Nioto Y, Kishi A et al. Role of active oxygen, lipid peroxidation and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut 1993; 34: 732 –737.
18. Banerjee S, Hawks C, Miller S, Dahill S, Beattie DV, McColl KE I. Effect of *Helicobacter pylori* and its eradication on gastric juice ascorbic acid. Gut 1994; 35: 317-322.
19. Olsen CE. Glutathione modulates toxic oxygen metabolite injury of canine chief cell culture in primary culture. Am J Physiol 1988; 254: 649-656.
20. Nasuti C, Gabbianelli R, Falcioni G, Cantalamessa F. Antioxidative and gastroprotective activities of anti-inflammatory formulations derived from chestnut honey in rats. Nutr Res 2006; 26: 130-137.
21. Rainsford KD. Gastric ulcerogenecity of non-steroidal anti-inflammatory drugs in mice with mucosa sensitized by cholinomimetic treatment. J Pharm Pharmacol 1978; 39: 669-672.
22. Whittle BJR. Temporal relationship between cyclooxygenase inhibition , as measured by prostacyclin biosynthesis and the gastrointestinal damage induced by indomethacin in the rat. Gastroenterol 1981; 80: 81-94.
23. Kauffman G. Aspirin induced gastric mucosal injury ; lesions learned from animal model. Gastroenterol 1989; 96: 606-614.
24. Hudson N, Hawthorne AB, Cole AT, Jones PD, Howley CJ. Mechanism of gastric and duodenal damage and protection. Hepatogastroenterol 1992; 39: 31.