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ANTI ULCER ACTIVITY OF ETHANOLIC EXTRACT OF ANNONA SQUAMOSA LEAVES

Ashokkumar Palanisamy*¹,
V. Rajesh², Perumal Perumal³, Billa Vamsi⁴

¹J.K.K.Nattraja College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu

²J.K.K.Nattraja College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu

³J.K.K.Nattraja College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu

⁴J.K.K.Nattraja College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu

ABSTRACT

The anti-ulcer activity of ethanolic extract of Annona squamosa leaves was investigated on aspirin induced ulcer models and pylorus ligation in wistar rats. In both models the common parameter determined was ulcer-index. Ethanolic extract of dosage 50, 100 mg/kg p.o produced significant inhibition of gastric lesions induced by pylorus ligation and aspirin induced ulcers. The extract 50mg/kg & 100mg/kg showed significant ($p<0.01$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicate that Annona squamosa leaves extract have potential anti ulcer activity in both models. This results may further suggests that ethanolic extract was found to posses antiulcerogenic as well as ulcer healing property, which might be anti secretory activity.

Key words: *Annona squamosa Linn*, antiulcer, aspirin, pylorus ligation.

Correspondence to Author

Ashokkumar Palanisamy

J.K.K.Nattraja College of Pharmacy,
Komarapalayam, Namakkal, Tamil
Nadu

Email: snm.manjula@gmail.com

INTRODUCTION

Herbal plants (cheaper accessibility and with fewer or no side effects) herb defined as any part of a plant which can be used for medicine, cooking, cosmetic uses and as a scent or dye. Herb plants produce and contain a variety of chemical substances that act upon the body. This plant are used to prevent, relieve, and treat illness. From a "scientific" perspective, many herbal treatments are considered experimental. The reality is,

however, that herbal medicine has a long and respected history. The current worldwide trend towards utilization of plant-derived remedies has, therefore, created a dire need for accurate and up-to-date information on the properties and uses, efficacy, safety and quality of medicinal products. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine (an antitumor), digitalis (a heart regulator), and

ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. (Kokate C.K., 1995). There is a worldwide 'green revolution, (Mukherjee, P.K., 2002) which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this upsurge of interest in plants is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin.

The plant *Annona squamosa* Linn. Belongs to Annonaceae family. It is small, semi-deciduous tree, found throughout India. It is traditionally used in anti-tumor, anti-diabetic and anti-lipidaemic activity. The bark can be used stop diarrhea in children and adults. Fruit is used to make a hair tonic. The plant is reported to contain 1-tritriacontanol (1) (17), (+)-o-methyl armepavine (2) (15), N-methyl corydaldine(3) (18), lanuginosone (4) (19,20), (+) anomuricine (5)(21), isocorydine (6) (22), N-methyl-6,7-dimethoxy isoquinolone (7)(23), 6,7-dimethoxy-2-methyl iosquinolinium (8) (24,25), β -sitosterol (9) and β -sitosterol-3-O- β -D-glucopyranoside (10) (26), 1-(4- β -D-glucopyranosyloxyphenyl)-2-(β -D-glucopyranosyloxy)-ethane (11) (26) and Rutin(Dinesh.K and Yadav,2011).

Gastric hyperacidity and ulcer are very common cause of human sufferings today. Although prolonged acidity, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still poorly understood(Rao et al.,2004). The present work attempts to evaluate the antiulcer potential of *Annona squamosa* Linn.

MATERIALS AND METHODS

This study was conducted in the pharmacology laboratory, department of pharmacology, J.K.K Nattraja College of Pharmacy, Komarapalayam, India.

Collection and extraction: the fresh leaves of *Annona Squamosa* L were collected from local area at komarapalayam, Tamilnadu. The material was taxonomically identified, confirmed and Available online on www.ijprd.com

authenticated by Botanical survey of India (BSI) at TN agricultural university, Coimbatore

The collected leaves were shade dried and the dried material was crushed to coarse powder with mechanical grinder. The powder was stored in air-tight container which was used for extraction. About 300 gm of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 18 hours till the solvent became colourless. At the end of the extraction process the marc was taken out and it was dried. After drying, the powdered marc was weighed & again packed and extracted with ethanol for another 72 hours till it became colourless. After that extract was concentrated by distillation. The final solution was evaporated, to obtain a syrupy greenish mass.

Preliminary phytochemical screening of extracts:

Qualitative chemical tests were conducted for ethanolic extracts to identify the various phytoconstituents employing standard screening tests(Kokate,2002). Ethanolic extract gave positive test for steroids, saponins, tannins, phenolic compounds and flavonoids.

Animals: Swiss albino mice of female sex weighing 20-25gms were used for the study. The animals were obtained from Agricultural University, Mannuthy, Trissur and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions ($25^{\circ} \pm 2^{\circ}\text{C}$; 12hr light and dark cycle). The animals were fed with standard diet and water *ad libitum*. Ethical clearance was obtained from the Institutional Animal Ethical Committee before performing the study on animals were taken for conducting antiulcer activities.

Acute toxicity studies: Acute oral toxicity study for ethanol extract of *Annona squamosa* leaves was carried out as per OECD guideline 425.

Aspirin induced ulcer:

Male albino-Wistar rats were divided in to four groups as mentioned above of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy.

Group I - control.

Group II - received 50mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Group III - received 100mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Group IV - received 20mg/kg, p.o omeprazole as standard.

Group was kept as control without any treatment. One hour after the drug treatment, the animals were treated with aspirin 200mg/kg by p.o, to induce ulcers. The animals were sacrificed after 4hrs and stomach was opened and percentage inhibition of ulcer was determined. (Kannappan *et al.*, 2008, Panda *et al.*, 1993, Parmar NS *et al.*, 1991, Pati K.S. *et al.*, 2008).

Pylorus ligation model:

The animals were divided in to four groups of six animals each as mentioned above.

Group I - received 1% CMC (1.0ml/kg p.o) as vehicle control

Group II - received 20mg/kg, p.o Omeprazole as standard

Group III - received 50mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Group IV - received 100mg/kg, p.o ethanol extract of *Annona squamosa* leaves.

Animals in all the groups were fasted for 36 h after the respective assigned treatment and were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and pylorus portion of stomach was lifted out and ligated. Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Four hours after the pyloric ligation, the animals were sacrificed by an excess dose of ether. The stomach was carefully removed and the gastric contents were collected. The gastric juice was centrifuged at 1000rpm and gastric volume was measured. Free and total acidities of the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/ L /100 gms. The stomach was cut open along the greater curvature and pinned onto a soft board for evaluating the

gastric ulcers and to calculate ulcer index. Ulcer scoring is done according to the scale mentioned below. (Vogel *et al.*, 2002)

Effect on gastric secretion by pyloric ligation in rats: pyloric ligation in rats was carried out according to Rao *et al.*, (2004). Ulcer index was calculated and intensity of gastric lesions was assessed. The no of ulcer was noted and the severity recorded with the following scores (Malairajan *et al.*, 2007).

ULCER INDEX (UI)

0 – Normal colored stomach

0.5 – Red coloration

1 – Spot ulceration

1.5 – Haemorrhagic streak

2 – Ulcers

3 – Perforations

Percentage inhibition:

Percentage inhibition was calculated using the following formula. (Malairajan *et al.*, 2007)

$$\text{Percentage protection} = \frac{\text{Control(UI)} - \text{Test (UI)}}{\text{control (UI)}} \times 100$$

Statistical studies:

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnet's 't' test using Graph Pad Prism software. (Trail version) The mean values \pm SEM were calculated for each parameter.

RESULTS

Phytochemical screening: The preliminary Phytochemical screening of the extract of *Annona squamosa* leaves showed the presence of carbohydrates, alkaloids, sterols, flavonoids, saponins, tannins and phenolic compounds, Protein and amino acids. The various phytoconstituents present in the extract.

Acute toxicity studies (LD₅₀): There was no change in normal behavioural pattern of extract treated animals and no sign and symptoms of toxicity were observed during the observations which was done continuously for the first two hours and then observed upto 24 hours for mortality. The extract

was safe upto maximum dose of 2000mg/kg body weight.

Aspirin induced ulcer:

Significant ($p < 0.001$) decrease in ulcer score was produced by Omeprazole, extract 50 and 100mg/kg when compare to control. Extract 100mg/kg produced decrease in ulcer score comparable ($p < 0.01$) to that of Omeprazole.

Extract at 50 and 100mg/kg produced maximum decrease in ulcer score which was better ($p < 0.001$) than Omeprazole at both doses of ethanolic extract. The percentage protection against ulcer by Omeprazole, extract at 50mg/kg and 100mg/kg body weight were found to be 79.70, 53.28 and 65.41 respectively (Table 1).

Table No 1: Effect of *Annona squamosa* Leaf extract on Aspirin induced ulcers

Groups	Ulcer Index	% Protection
Group I Control	18.71±0.10	--
Group II Standard (Omeprazole)	3.79±0.07***	79.7 %
Group III <i>Annona squamosa</i> Extract 100mg	6.47±0.08***	65.41%
Group IV <i>Annona squamosa</i> Extract 50mg	8.74±0.08**	53.28 %

All values represent Mean ± SEM, n=6 in each group. *** $P < 0.001$, ** $P < 0.01$, Control group is compared with standard and extract doses.

Pyloric ligation induced gastric ulcer:

The ethanolic extract at both the doses at 50 and 100mg/kg body weight produced significant ($p < 0.001$) decrease in ulcer score when compare to control. Ethanolic extract at both doses at 50 and 100mg/kg body weight produced decrease in ulcer score comparable ($p < 0.01$) to that of Omeprazole.

The percentage protection against ulcer by Omeprazole, extract at 50 and 100mg/kg body weight were found to be 61.54, 27.25 and 40.77 respectively. The ethanolic extract at both the doses produced significant ($p < 0.001$) decrease in gastric volume, total and free acidity indicating its anti-secretory activity (table 2, 3).

Table No 2: Effect of *Annona squamosa* leaf extract on pylorus ligation induced ulcers

Groups	Ulcer Index	% Protection
Group I Control	14.64±0.10	--
Group II Std(omeprazole)	5.63±0.10***	61.54 %
Group III <i>Annona squamosa</i> Extract 100mg	8.67±0.07***	40.77%
Group IV <i>Annona squamosa</i> Extract 50mg	10.65±0.10**	27.25 %

All values represent Mean ± SEM, n=6 in each group. *** $P < 0.001$, ** $P < 0.01$, Control group (Group I) is compared with standard and extract doses.

Table No 3: Effect of *Annona squamosa* leaf extract on gastric secretions, free acidity and total acidity on pylorus ligation model

Group	Gastric Volume	pH	Free Acidity	Total Acidity
Control	5.56 ± 0.10	1.5 ± 0.08	65.06 ± 1.19	70.75 ± 1.21
Std(omeprazole)	1.46±0.07***	4.31±0.07***	28.95±0.96***	36.99±0.97***
Extract 100mg	2.64±0.07**	3.44±0.08***	45.71 ± 0.88**	54.04±1.11***
Extract 50mg	3.48 ± 0.01**	2.45 ± 0.09**	53.19 ± 0.54*	63.03±1.15**

All values represent Mean ± SEM, n=6 in each group. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.005$ Control group is compared with standard and extract doses.

DISCUSSION

Most of the studies demonstrate the importance of natural products in drug discovery. In these study antiulcer activity of Ethonolic extract of *Annona squamosa* has been studied. The antiulcer study was evaluated using aspirin and pylorus ligation models.

Most of the studies demonstrate the importance of natural products in drug discovery. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs. The acute oral toxicity study result showed that the plant leaf is safe.

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 disease (PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. It includes number of drugs such as proton pump inhibitors and H2 receptor antagonists are available for the treatment of peptic ulcer, Peptic ulcer occurs due to an imbalance between aggressive (acid, pepsin) and defensive (gastric mucosal barrier) factors of gastric mucosa.

Aspirin induced model shows significant percentage inhibition when compared with standard. As aspirin is COX inhibitors suppress gastro duodenal bicarbonates secretion and endogenous prostaglandin biosynthesis, disrupts mucosal barrier. The ulcer index parameter was used for the evaluation of ulcer activity. Moreover the disturbance of defensive factor like mucus secretion, bicarbonate secretion and mucosal blood flow has been reported to cause ulcer.

Pylorus ligation modelis usually employed to observe the potential of anti ulcer drugs for their anti-secretory activity by checking the gastric volume and its effect on gastric pH, total acidity and free acidity. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier and also because of an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion.

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CONCLUSION

It was found that antiulcer activity exhibited was due to mucosal defensive factor. Hence it can be used for management of peptic ulcer.

Chemical substances derived from plant have got a very long history in treatment of human diseases. Nearly 50% of new chemical entities introduced during the past two decades were from natural products.

Further research is required to isolate the active phytoconstituents present in the extract and experimentation on the healing action of drug on chronic ulcer as well as on the possible side effects. The investigation on mode of action may pave way for establishment of new anti-ulcer therapy regimen.

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