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Journal of Chemical and Pharmaceutical Research, 2016, 8(4):973-979

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of antiulcer and thrombolytic activity of aqueous extract of *Tiliacora acuminata* bark in albino wistar rats

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ABSTRACT

The study was carried out to find out the antiulcer activity and thrombolytic activity of aqueous extract of Tiliacora acuminate. For this study albino Wistar rats(150-200g) were used .Four groups of rats were selected containing six animals in each group. The study was carried on different gastric ulcer models and ulcers were induced by pyloric ligation and aspirin. The animals were treated with Omeprazole 20mg/kg and AETA 200 and 400mg/kg. Ulcer index was calculated in both models. In pyloric ligation model, free acid, total acid, and total protein were also determined. Thrombolytic activity also determined. AETA produced significant reduction in ulcer index (0.72) when compared with control (4.5) in both ulcer models. In pylorus ligated model it showed significant reduction in volume of acid secretion and increased mucin and total protein content .It also produced significant increase in Free radical scavenging and lipid peroxidation content. AETA produces significant antiulcer activity which is comparable to standard drug Omeprazole. It also produces significant Thrombolytic which is comparable to standard streptokinase.

Key words: Pyloric ligation, thrombolytic, streptokinase.

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries. [1] Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors. [2] In Ayurveda, peptic ulcer mostly refers to *Amlapitta*or *Parinamasula*. *Amlapitta* is a disease of the gastrointestinal tract, especially of the stomach. *Amlapitta*literally means, pitta leading to sour taste. [3] Number of drugs including proton pump inhibitors, prostaglandins analogues, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer.

But most of these drugs produce several adverse reaction, including toxicities and even may alter biochemical mechanisms of the body upon chronic usage. [4] Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors. [5-6]. *Tiliacora acuminata* is a stronger diuretic and heart tonic. [8] *Tiliacora acuminata* reported in the Siddha system as a remedy for jaundice, piles, ulcer, leprosy and blood purifier. [9-10]Chemically it contains Flavonoids (quercetin), Saponins, alkaloids, tannins and phenolic compounds. [11-12]The following activities like analgesic, larvicidal, Hepatoprotective, and hypoglycaemic were reported[13-16]

Recent screening of plants revealed many compounds like Flavonoids, alkaloids, Saponins, terpenoids, monoterpenoids (linalol), glycoprotein's, polysaccharides, tannins, essential fatty acids, phenolic compounds and vitamins having pronounced antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potential.[17] Scientific literature is continuously reporting herbal drugs having anti-ulcer potential. There is need to evaluate the potential of ayurvedic remedies as adjuvant to counteract side effects of modern therapy. The present investigation is aimed at studying the anti-ulcer activity of the aqueous extract of bark of *Tiliacora acuminate.*in order to justify the traditional claims endowed upon this herbal drug as a rasayana.

EXPERIMETAL SECTION

Plant Material

The bark of *Tiliacora acuminata.* were collected at in the area of Srisalium, India and authenticated by Dr. K. Ravikanth of the Department of Botany, DNR Degree and P.G.College, Bhimavaram, voucher specimen are for future reference.

Extraction

Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat- stable constituents. This process is typically used in preparation of Ayurvedic extracts called "quath" or "kawath". The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.

Preliminary Phytochemical Screening

Aqueous extract of *Tiliacora acuminate* was subjected to preliminary phytochemical for the detection of various constituents. [18]

Experiment

Albino Wistar rat of weighing between 200-250 g was housed in groups of 5 to 6. All rats were feed with pelleted diet (Pranav Agro Industries Ltd, Sangli, India) and water ad libitum. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA,

IAEC NO:**439/PO/01/a/CPCSEA**

All the drugs(Omeprazole, Aspirin) and chemicals(Ethanol, Toffer reagent and phenopthelin) used are of analytical grade .

Acute Toxicity Studies

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines 429. [19] Animals were divided in groups (n=4). And were fasted for 4 h. with free access to water only. The AETA extracts was administered orally in doses of 5,50,300 and 2000 mg/kg to different groups of mice and observed over 24 hr. for mortality and physical/ behavioural changes.

Assessment of Anti-Ulcer Activity

Animals were divided into 4 groups each containing six in number.
Group I: Control received only Distilled Water .
Group II: Standard received Omeprazole (20 mg/kg).
Group III: Received Aqueous extract of *Tiliacora acuminata.* (200mg/kg)
Group IV: Received Aqueous extract of *Tiliacora acuminata*.(400 mg/kg).

Evaluation parameters:(20) (21)
Collection of Gastric juice
Ulcer Scoring:
Free acidity and Total acidity:
Determination of total protein content**:**

Thrombolytic activity

The thrombolytic activity of all extractives was evaluated by the method14 using streptokinase as standard. The dry crude extract (100 mg) was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered. Aliquots (5 ml) of venous blood were drawn from healthy volunteers which

were distributed in five different pre weighed sterile micro centrifuge tube (1 ml/tube) and incubated at 37 °C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone). To each micro centrifuge tube containing pre-weighed clot, 100 µl aqueous solutions of different partitionates along with the crude extract was added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released of fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown Below: (22)[23]

% of clot lysis = (wt of clot after lysis /clot wt) × 100

Statistical analysis:

The results were expressed as mean ± SEM (n=6) .Statistical analysis was performed using one way ANNOVA followed by Dunneth comparison test T.P-values (expressed) calculated against Ulcer group and $p<0.001$ were considered.

RESULTS

Preliminary Phytochemical Screening

The AETA were found to contain carbohydrates,, amino acids, Saponins, Flavonoids, tannins and phenolic compounds.

Acute Oral Toxicity Study

Table 1

Dose	Behavioural studies	Mortality
5mg/kg	Normal	Nil
50 mg/kg	Normal	Nil
300 mg/kg	Normal	Nil
2000 mg/kg	Normal	Nil

- *General behavior of the animals was observed at 1,3 and 24 hr after administration.*
- *No mortality was observed and hence the extract falls under the category V.*

Pyloric Ligation Induced Gastric Ulceration

Table 2

Groups	Gastric volume (ml)	PH	Free acidity	Total acidity	Mean ulcer index	%protection
Group I (control)	9.5±0.02	2.5±0.2	102±0.2	115±0.55	4.5±0.33	_
Group II (standard)	5.2±0.5***	4.2±014	31±0.22***	39±0.44***	0.84±0.36***	82.8±0.15
Group III (test I)	6.8±0.04**	3.2±008±	60±0.23**	78±0.25**	0.50±0.55**	50.5±0.21
Group IV (test II)	5.5±05**	3.7±0.2	65±06**	65±0.55**	0.72±0.55**	72.5±0.21

Aspirin induced gastric ulceration

Table 3

Groups	Gastric volume (ml)	PH	Free acidity	Total acidity	Mean ulcer index	%protection	Total protein content
Group I (control)	1.5±0.05	2.5±0.45	116±0.16	120±0.55	5.5±0.13	_	8.34±0.24
Group II(standard)	0.7±0.13***	4±0.32	32±0.8***	42±0.8***	0.84±0.01***	84.8±0.13***	14.50±0.17
Group III (test I)	1.2±0.12**	3±0.35**	65±0.44**	70±0.25**	0.63±0.1**	63.6±0.21	10.50±0.50
Group IV (test II)	0.9±0.13***	3.8±0.16	55±0.12**	59±0.06**	0.68±0.00***	68.88±0.06**	12.48±0.17

Thrombolytic activity :

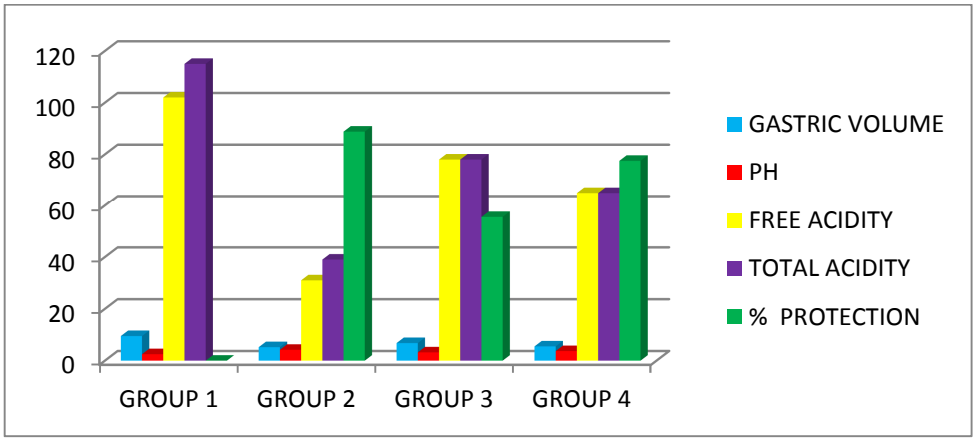
Table 4

Sample	Thrombolytic activity (% Clot lysis)
Streptokinase	70.4±1.20***
AETA	10±0.46*
META	32±0.84**

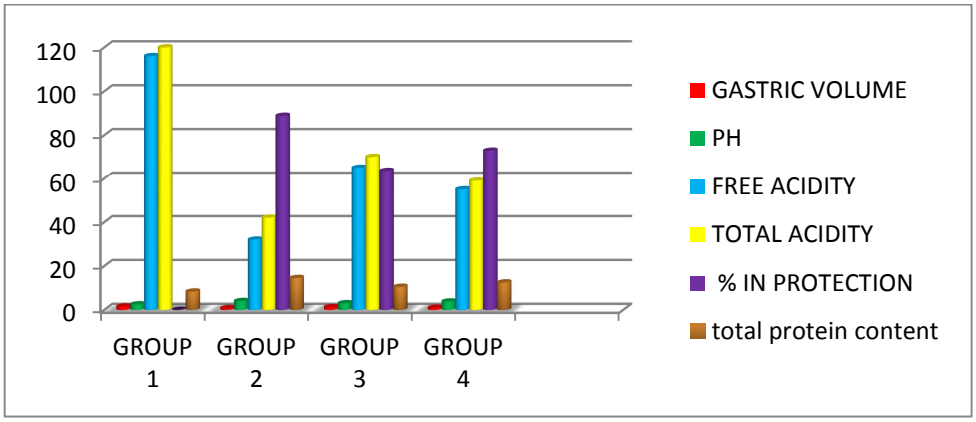
Values are expressed as Mean ± S.E.M.
One way ANOVA followed by Dunnet'st – test .
****p< 0.01, **p< 0.05, *p< 0.1 as compared to control group.*

Pyloric Ligation Induced Gastric Ulceration

Graph 1

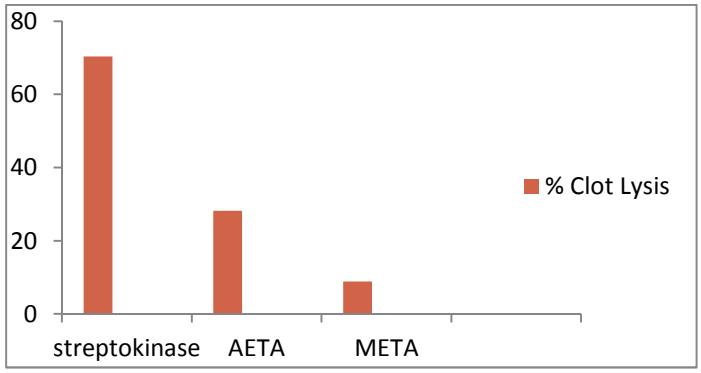


Aspirin induced gastric ulceration
Graph 2

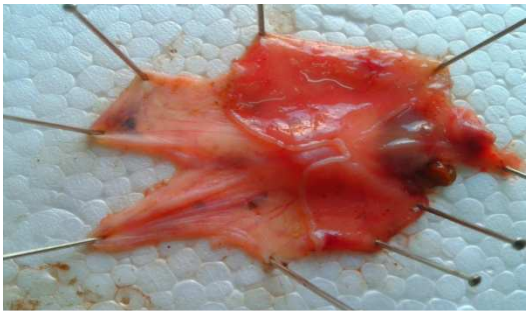


Thrombolytic activity

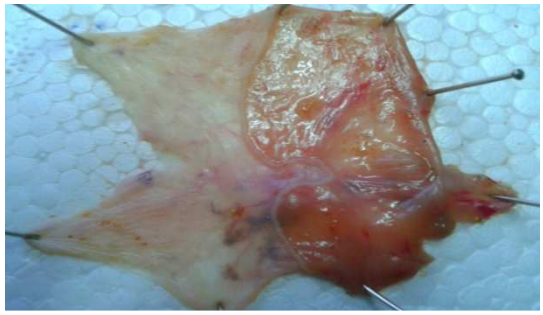
Graph 3



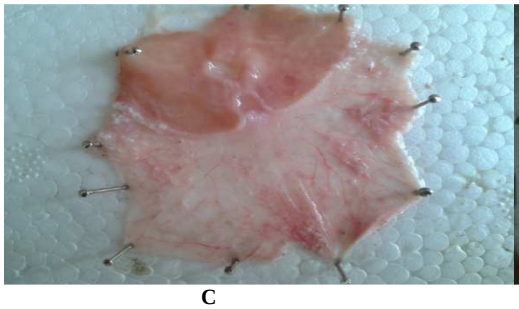
MICROSCOPICAL VIEW OF RAT STOMACH
Pyloric ligation method



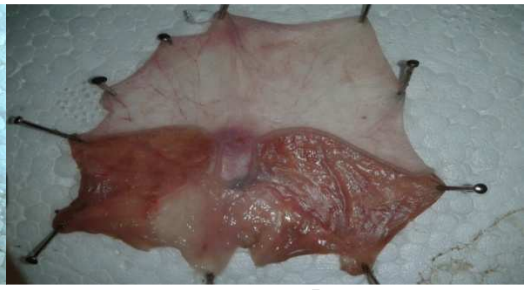
A
Group I control



B
GroupII (Omeprazole 20mg/kg)



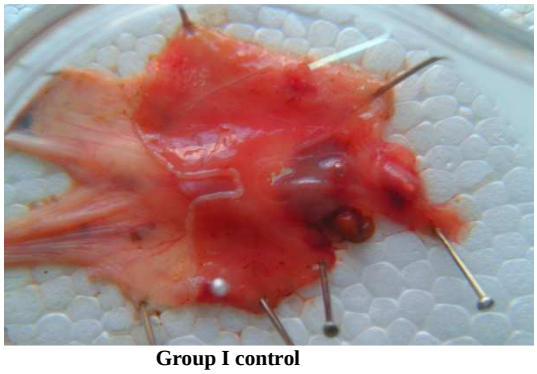
C
Group III (AETA200mg/kg)



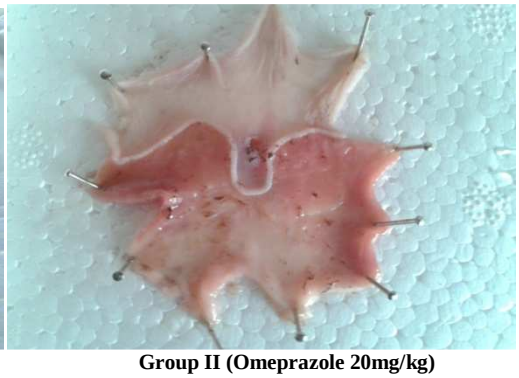
D
Group IV (AETA 400mg/kg)

Microscopically view of rat stomach

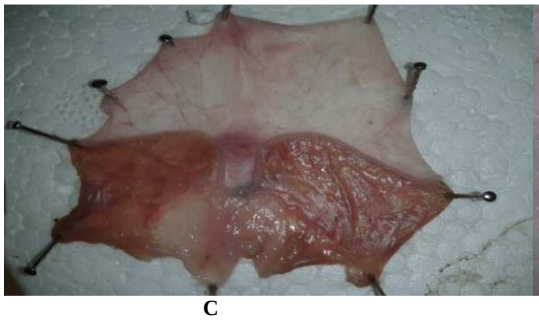
Aspirin induced gastric ulceration



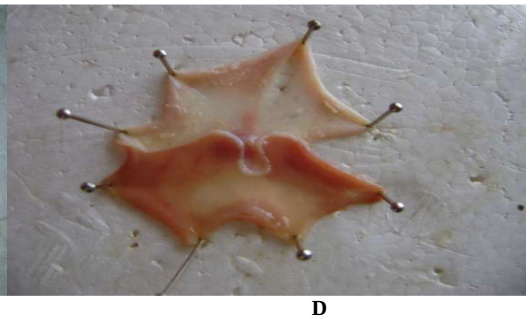
Group I control



Group II (Omeprazole 20mg/kg)

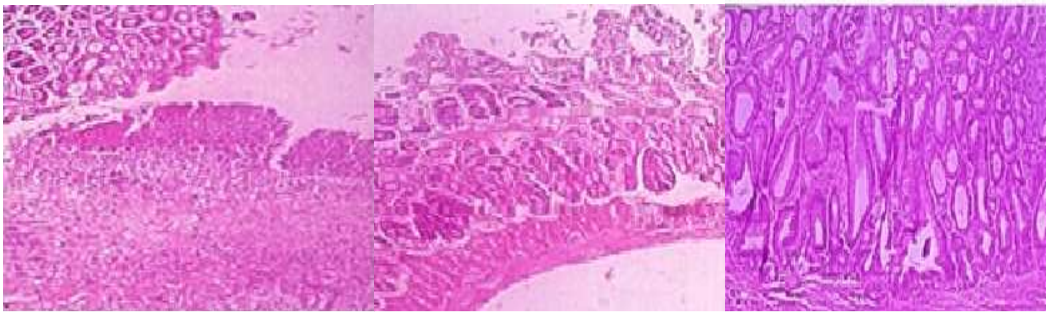


C
Group III (AETA 200 mg/kg)



D
Group IV (AETA400mg/kg)

Histopathology results of Aqueous Extract *Tiliacora acuminata* bark of in pyloric ligation induced ulcers



A **B** **C**

A) Control: Rat stomach showing severe ulcer lesions and desquamation of the surface epithelium in pyloric ligation gastric ulcers.
B) Standard: Rat stomach fairly protected with Omeprazole (20mg/kg) in pyloric ligation induced ulceration.
C) AETA(400mg/kg): Rat stomach showing a protected epithelium due to Aqueous extracts of T.accuminata(400mg/kg) in pyloric ligation induced gastric ulceration.

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents including plant extracts may be used.Aqueous extract of *Tiliacora acuminata*.is one such herbal drug used in the present study primarily to evaluate the anti-ulcer genic in pylorus ligation and aspirin induced ulcers in rats. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and 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. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and haemorrhage.

Aqueous extract of *Tiliacora acuminata* of anti ulcer genic activity of APTA was studied in aspirin induced gastric mucosal damage model in Swiss albino rats. This model was chosen because NSAID abuse is the main exogenous cause of refractory peptic ulcer constituting 39% of the cases of peptic ulcer. NSAIDs produce a spectrum of injury to the gastrointestinal mucosa, from hemorrhages and petechial to erosions and ulcers. Aspirin is known to inhibit PG cyclooxygenase, leading to reduced production of PGE and endothelial PGI. This causes vasoconstriction, inhibition of platelet aggregation (enhanced bleeding) and contributes to the enhanced acid secretion. It can also cause mast cell degranulation resulting in the release of histamine. Tissue damaging free radicals which are produced from the conversion of hydroperoxy to hydroxy fatty acids further contribute to cell destruction. In our study Aqueous extract of *Tiliacora acuminata*.significantly reduced the ulcers induced by aspirin and results were comparable with omeprazole.

CONCLUSION

Among the two doses (200 and 400 mg/kg) of 400mg/kg Aqueous extract of *Tiliacora acuminata*, produces significant antiulcer and thrombolytic activity.

Acknowledgment:
The authors are thankful to the Principal, Chairman and management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowadavaram, Guntur, for providing the facilities for conducting the study.

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