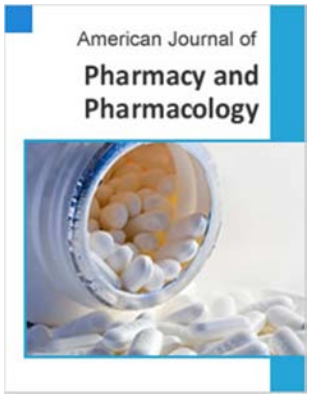
American Journal of Pharmacy and Pharmacology
2017; 4(5): 41-44
http://www.aascit.org/journal/ajpp
ISSN: 2375-3900

American Association for Science and Technology



Keywords
Antiulcer Activity,
Daemia extensa,
Ulcer Index,
Percentage Protection,
Omeprazol,
Pylorus Ligation

Received: April 10, 2017
Accepted: April 27, 2017
Published: August 23, 2017

Evaluation of Antiulcer Activity of Ethanolic Extract of *Daemia extensa* (Jacq.) R. Br. Seed

Dheeraj Jain[1, *], Sachin Kumar Jain[2], Praveen Sharma[2], Priya Jain[3], Nitesh Jain[3]

[1]Department of Pharmacognosy, Shri NMT Gujrati College of Pharmacy, Indore Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, India
[2]Department of Pharmacognosy, College of Pharmacy, IPS Academy, Indore, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, India
[3]Department of Pharmacy, Oriental University, Indore, India

Email address

dearestdk@yahoo.com (D. Jain)
[*]Corresponding author

Citation

Dheeraj Jain, Sachin Kumar Jain, Praveen Sharma, Priya Jain, Nitesh Jain. Evaluation of Antiulcer Activity of Ethanolic Extract of *Daemia extensa* (Jacq.) R. Br. Seed. *American Journal of Pharmacy and Pharmacology*. Vol. 4, No. 5, 2017, pp. 41-44.

Abstract

Daemia extensa is a well-known traditional plant in India. The plant is having laxative, anthelmintic, astringent, Immunosuppressive, potent antioxidant, *in-vitro* antibacterial and antifungal activity. The aim was to search for anti-ulcer activity of ethanolic extract of *Daemia extensa* seed. Ethanolic extract of *Daemia extensa* seed was considered for its potential to protect gastric mucosa against ulcers induced by pylorus ligation and indomethacin induced methods. Ulcer index, free acidity, total acidity and percentage protection were used for evaluation of anti-ulcer activity. Pre-treatment with *Daemia extensa* seed extracts in dose of 400 mg/kg in both the models significantly diminished the ulcer index and the percentage protection of ulcer compared with control group ($p<0.05$). Based on the studies we concluded that the ethanolic extracts of *Daemia extensa* seeds might contain some active principles against ulcer healing properties.

1. Introduction

Peptic ulcer disease is a very common global health problem today. Peptic ulcer is a lesion of gastric or duodenal mucosa. Duodenal ulcers are more common in adult males. Gastric ulcers occur commonly at old age and in lower socio-economic class of individuals. Although the exact cause of ulceration is not known, hydrochloric acid and pepsin are responsible for maintaining the lesion once it is produced. Peptic ulceration occurs only in areas, which are bathed by the acidic gastric juice. Therefore, the term peptic ulcer refers to ulceration of the areas which might be acted upon by acid peptic juice namely the stomach and the first portion of duodenum [1]. Peptic ulcers also occur at the lower end of the esophagus, on the jejunal side of a gastroenterostomy and in Meckel's diverticulum [2].

The current therapeutic approach to gastric ulceration is to achieve inhibition of gastric secretion, promotion of gastric protection, blockage of apoptosis, and epithelial cell proliferation for effective healing [3]. In recent years, focus on plant research has in-creased worldwide and several studies had showed immense

potential of medicinal plants. Herbal medicines derived from plant extract, are increasingly being recognized in treating various clinical diseases, with relatively little knowledge of their modes of action [4].

In ancient system of medicines, herbal preparations were used for treating duodenal ulcers [5]. In the last few years, efforts have been taken to identify new antiulcer drugs from natural sources like plants [6].

The plant *Daemia extensa* (Family Asclepiadaceae) is commonly known as Chagulbati in Hindi. A perennial twining fetid herb with white latex. Stems clothed with spreading hairs. Leaves 5-10 cm long, broadly ovate or sub orbicular, acuminate, velvety pubescent beneath, base deeply cordate. Flowers greenish yellow or dull white, in lateral cymes. Follicles deflexed, 5-7.5 cm long, lanceolate, attenuated into a long beak, echinate with soft spines. This plant is considered a native of India especially available in Khandesh and Nagpur-Wardha division in Maharashtra and in Andhra Pradesh [7]. Traditionally the plant is reported to have antiulcer activity [8]. In the present study, an effort has been made to establish the scientific alidity to the antiulcer property of the bark extracts of *Daemia extensa* in aspirin induced ulcer in male albino rats.

2. Materials and Methods

Preliminary phytochemical investigation

Preliminary phytochemical investigation of ethanolic extracts of bark of *Daemia extensa* revealed the presence of phenols, glycosydes, carbazole, alkaloids, flavonoids and tannins [9].

Collection of plant materials

Fresh seeds of *Daemia extensa* were collected locally from the Indore district of Madhya Pradesh and identified by Department of Botany, Government Degree College, Indore and submitted to department. The seeds were shade dried and were crush to moderately coarse powder.

Preparation of extract

The freshly collected seed were dried under shade, sliced into small pieces, pulverized using a mechanical grinder and passed through 40 mach sieve, and preserved in air tight container for further use. The powdered seed were extracted with 95% ethanol. After exhaustive extraction, the extract was concentrated by distillation process. A brownish black colored residue was obtained (yield 19.8% w/w), which was kept in a desiccators. This ethanolic extract of *Daemia extensa* seed (DES) was used in further experiments.

Experimental Animals

Albino Wistar rats of both sex weighing between 150-250 g were used. The experimental protocol was approved from Institutional Animal Ethics Committee. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water *ad libitum.*

Acute toxicity studies

Acute toxicity was carried out as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Organization for Economic Cooperation and Development (OECD). Group of three rats weighing between 22-30 gm were selected and kept for 3-4 hrs fasting with free access to water. Doses were calculated according to body weight and seed extracts were dissolved in rice bran oil and administered orally at a starting dose of 2000mg/kg and were observed for 24 hours.

Pylorus ligation Induced Ulcers [16]

All animals were divided into four groups of six animals each. The groups were treated as follows:

Group I - Control (saline solution) p.o.

Group II - Reference standard (Omeprazole 20 mg/kg) p.o.

Group III - Ethanolic seed extract of DE (200 mg/kg, p.o.)

Group IV - Ethanolic seed extract of DE (400 mg/kg, p.o.)

After 1 hr of treatment to different groups, the animals were anaesthetized using thiopentone sodium (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage in its blood supply. After 4 hr their stomachs were dissected and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity. The percentage of ulcer protection was calculated.

$$\text{Percent Protection} = (C-T/C) \times 100$$

Where C= ulcer index in control group

T= ulcer index in treated group

Collection of Gastric Juice

Gastric juice was collected from pylorus-ligated rats as mentioned earlier. Collected gastric juice centrifuged for 1000 rpm for 10 minutes and the volume of gastric juice was measured. This gastric juice was used for biochemical estimations as follows.

Determination of Free Acidity and Total Acidity

Reagents

1. Newly prepared 0.01N oxalic acid solution for standardization of sodium hydroxide
2. Newly prepared 0.01 N NaOH solution
3. Topfer's reagent: It is dimethyl amino benzene 0.5% in absolute ethanol
4. Freshly prepared 1% phenolphthalein solution

Procedure

1. Gastric juice (1ml) was occupied in to a 100 ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01 N sodium hydroxide until all traces of red color disappears and the color of the solutions turns yellowish orange (end point).
2. The volume of alkali added was noted. This volume resembles to free acidity.
3. Then 2 to 3 drops of phenolphthalein solution was mixed and titration was continued until a definite red tinge reemerges.
4. The volume of alkali added was noted which resembles to total acidity [10]

Acidity was designed by using the formula:

Acidity = Volume of NaOH x Normality of NaOH/ 0.1 X 100 mEq/litre

Indomethacin induced ulcer

All animals were separated into four groups of six animals all. The groups were treated as follows:

Group I - Control (saline solution) p.o.

Group II - Reference standard (Omeprazole 20 mg/kg) p.o.

Group III - Ethanolic seed extract of DE (200 mg/kg, p.o.)

Group IV - Ethanolic seed extract of DE (400 mg/kg, p.o.)

The test drugs were overseeing orally in 2 % Acacia solution 10 min prior to oral indomethacin in a dose of 20 mg/kg. Six hours later, the rats were detriment in diethyl ether anesthesia and their stomachs were detached. Formal-saline (2% v/v) was then injected into the much ligated stomachs for storing overnight. The next day, the stomachs were unbolted along the Greater curvature, then eroded in warm water, and examined under a 3- fold magnifier. The distances of the longest widths of the lesions were measured and summated to give a total lesion score (in mm) for every animal, then the mean count for every group being calculated.

Statistical Analysis

The data are characterized as mean ± S.E.M, and statistical significance was carried out retaining one way analysis of variance (ANOVA) followed by Dunnett t-test where $p<0.05$ was considered statistically significant using Graph pad 5 software.

3. Results and Discussion

Pylorus ligation Induced ulcers

The gastric secretion determination model used ligated pylorus, treatment with *Daemia extensa* extracts (200 and 400 mg/kg) and OMZ (20 mg/kg) correspondingly, condensed the volume of gastric juice, free acidity, total acidity and raised gastric pH significantly in comparison with the control group (Table 1 & Figure 3).

Table 1. *Gastroprotective activity of ethanolic extract of Daemia extensa on pylorus ligated Ulcer.*

Treatment	Dose	Mean ulcer index*	% protection	Gastric Juice ml	pH of gastric juice	Free acidity meq/ltr	Total acidity meq/lt
Vehicle	1ml/100g	11.07±3.14	--	4.5±0.34	1.7±0.08	28.55±2.33	43.35±0.13
Omeprazole	20mg/kg	3.05±0.74**	82.60%	2.08±0.12	4.8±0.07	10.04±0.06	29.55±0.11
EEDE	200mg/kg	3.56±1.25**	66.95 %	4.98±0.16	3.35±0.15	19.05±0.22	37.24±0.34
	400 mg/kg	4.68±0.76**	73.04 %	5.05±0.18	4.68±0.08	21.17±0.12	38.55±0.06

*Mean ± SEM (n = 6). Significant at **$p<0.01$ compared to control group.

This study revealed a noteworthy antiulcer activity of the ethanolic extracts of *Daemia extensa* using pylorus ligation and indomethacin induced ulcer models in rats. The etiology of peptic ulcer is unidentified in most of the cases, however it is normally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms [11]. To regain the equilibrium, different therapeutic agents are used to constrain the gastric acid secretion or to boost the mucosal defense mechanisms by growing mucosal production, stabilizing the surface epithelial cells or intrusive with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are supposed to be due to stress-induced upsurge in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is an significant factor in the formation of ulcer due to contact of the unprotected lumen of the stomach to the accumulating acid [12]. Pylorus ligation induced ulcer was used to study the effect of extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach grounds accumulation of gastric acid in the stomach. This upsurge in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model contains fasting of rats for overnight trailed by ligation of pyloric end of the stomach. The ulcer index is determined 19 hours after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach [13].

Indomethacin induced ulcer

There was an enlightened decline in ulcer index of the rats pretreated with the extract. The deterioration was significant ($P<0.05$-0.01) associated to control. However, the reduction of ulcer index caused by the standard drug, Omeprazole (20 mg/kg) was higher than that of the extracts (Figure 4). Indomethacin is known to cause ulcer especially in an empty stomach [14] and typically on the glandular (mucosal) part of the stomach [15, 16] by constraining prostaglandin synthetase through the cycloxygenase pathway. Prostaglandins purpose to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair. Suppression of prostaglandins synthesis by indomethacin results in upsurge defenselessness of the stomach to mucosal injury and gastroduodenal ulceration. The extract was experiential to significantly reduce mucosal damage in the indomethacin induced ulcer model, signifying the possible extract mobilization and participation of prostaglandin in the anti-ulcer effect of the extract [18].

Macroscopical Evaluation

Stomachs were pinned on a flat surface and examined with a binocular dissector microscope at 10X magnification. Macroscopical change of pylorus ligation and indomethacin induced ulcer models were shown in Figure 1 and Figure 2.

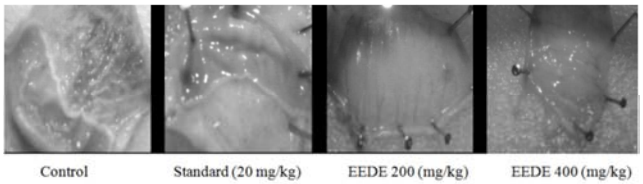


Figure 1. *Healing of Pylorus ligated Induced gastric ulcer.*

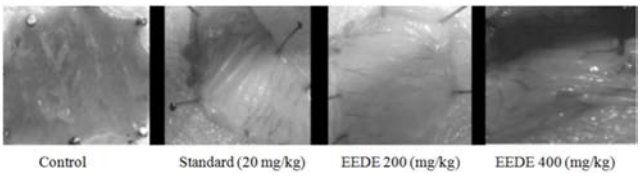


Figure 2. *Healing of Indomethacine induced gastric ulcer.*

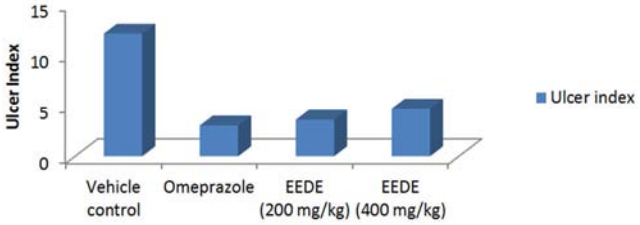


Figure 3. *Effects of Daemia extansa seed extract, control and standard group on ulcer index in pylorus ligated ulcer.*

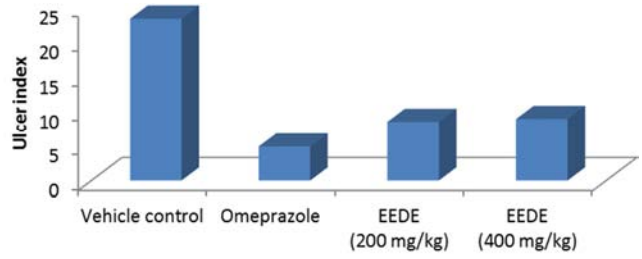


Figure 4. *Effects of Daemia extansa seed extract, control and standard group on ulcer index in indomethacin-induced model.*

4. Conclusion

The ethanolic extract of *Daemia extensa* seeds presented a protective effect against gastric ulcers induced by pylorus ligation and indomethacin. The current study established that *Daemia extensa* seed extracts at a single dose of 400 mg/kg suggestively reduced the ulcer index compared with control group ($p<0.05$).

References

[1] S. F. D. Andrade, M. Lemos, E. Comunello, V. F. Noldin, V. C. Filho, R. Niero. *Journal of ethnopharmacol.* 113: 252-257, 2007.

[2] N. Begum, C. Mayuren, N. Balaji, R. Y. Chinnapa, K. Aravind. *Adv. Pharmacol. Toxicol.* 9(3): 33-36, 2008.

[3] P. Malairajan, G. Gopala Krishnan, S. Narasimhan, K. Jessika-laveni. *Indian J Pharmacol*. 40(3):126-128, 2008.

[4] W. A. Boyd. *Text Book of Pathology Structure and Function in Disease*. (2nd ed.). United Kingdom: Lea & Febiger, 1970.

[5] T. K. Chatterjee. *Herbal options*. Calcutta: Books and Allied Private Limited, 2000.

[6] E. O. Jude, A. Paul. *J. Pharm. Sci.* 22: 384-390, 2009.

[7] N. C. Dey, T. K. Dey *A Text Book of Pathology*. (5th ed.). Culcutta: New Central Book Agency, 2002.

[8] V. Kanta, S. L. Bodhankar, Y. V. Machave, P. A. Thakurdesai. *Adv Pharmacol Toxicol*. 7(1): 83-87, 2006.

[9] K. R. Khandelwal. Practical Pharmacognosy. Techniques and experiments. 2nd ed. Pune: Nirali Prakashan, 149-56, 2000.

[10] S. Edwin, J, Edwin, L. Deb, S. Goyal, S. Gupta. 44(5): 395-397, 2007.

[11] A. Mohammed, J. Ravikumar, H. Y. Santosh, M. N. Nagashruthi. Indian Drugs 45(12): 979-981, 2008.

[12] P. B. Hawk, B. L. Oser, H. W. Summerson. *Practical physiological chemistry*. (12th ed.). London: Churchill; 1947.

[13] C. K. Kokate, A. P. Purohit, S. B. Gokhale. *Pharmacognosy*. (20th ed.). Pune: Nirali Prakashan, 2009.

[14] J. P. Mohanty, L. K. Nath, N. R. Bhuyan, U. K. Nayak. Int J Pharmaco. Biol Sci 2(1): 159-164, 2008.

[15] J. E. Okokon, P. A. Nwafor. Pakistan Journal of Pharmaceutical Sciences 22: 384-90, 2009.

[16] P. H. Patil S. J. Surana Int J Pharmacol Biol Sci 3(1): 81-93, 2009.

[17] D. Raju, K. Ilango, V. Chitra, K. Ashish. Journal Pharmaceutical Sciences and Research 1: 101-7, 2009.

[18] R. K. Srinivas, K. Srisailam, V. M. Reddy, Int J Pharmacol Biol Sci. 3(1): 51-54, 2009.