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Evaluation of antiulcer activity of roots of *Acacia catechu* Willd. (Mimosoideae)

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Abstract

The present study was conducted to evaluate the antiulcer activity of 95% ethanolic and aqueous extract of roots of *acacia catechu* willd. *Acacia catechu* willd. (Mimosoideae) is a deciduous, thorny tree of genus *Acacia* which grows upto 15 m height; widely distributed throughout India and used traditionally for the treatment of asthma, bronchitis, pain in chest, ulceration, wound healing, sores in mouth, colic, vitiligo, cancer, sore throats, diarrhea, antiviral, spasmolytic, antifungal and hypoglycemic activity. Previous investigations of some other *Acacia* species revealed that the genus *acacia* be a rich source of flavonoids and tannins, which exhibited broad activities including antioxidant, antipyretic, antileprosy, antidiarrheal, antimicrobial, antibacterial, antifertility, anticancer, immunomodulatory, chronic renal failure, hepatoprotective, hypotensive, hypoglycemic & wound healing. Antiulcerogenic activity of this drug has been evaluated using aspirin + pylorus ligated ulcer model, ethanol induced ulcer model and NSAID's induced ulcer model. Both extract of roots of *Acacia catechu* Willd found to possess antiulcer activity and it had observed that activity increases as the dose of extracts increases.

Keywords: Antiulcer, *Acacia catechu* Willd., Aspirin + Pylorus ligated ulcer model, Ethanol induced ulcer model, NSAID's induced ulcer model.

1. Introduction

Peptic ulcer is the most common ulcer found in the human beings. Peptic ulcer, also known as *ulcus pepticum*, PUD or peptic ulcer disease, is an ulcer (defined as mucosal erosions equal to or greater than 0.5 cm) of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. About 80% of ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach. Ulcers can also be caused or worsened by drugs such as aspirin and other NSAIDs^[1-2].

Reports on clinical evaluation of allopathic drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy. Hence, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse^[3]. The present study conducted for the same purpose.

Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae. *Acacia catechu* Willd. common names include Catechu, Cachou and Black Cutch^[4]. *Acacia catechu* Willd. is widely distributed throughout the Sub-Himalayan tract of Punjab to Assam ascending to 1200 m, peninsular region, particularly in drier parts, Madhya Pradesh, Maharashtra, Gujarat, Bihar, Rajasthan, Tamil Nadu, eastern slopes of Western Ghats, Ganjam, Burma, throughout the Konkam, S.M. country & Deccan^[5].

catechu Willd. plant is traditionally used for asthma, bronchitis, pain in chest, ulceration, wound healing, sores in mouth, colic, vitiligo, cancer, sore throats and diarrhea and it also possess antiviral, spasmolytic, antifungal and hypoglycemic activity. Leaves are used in gonorrhoea; flowers for dysentery and root for rheumatism, toothache, anti-bacterial and fungicidal activity. The saline extract of seeds shows leukoagglutinating activity against leukaemic cells. Bark is astringent and abortifacient, used for scabies & chronic skin troubles, childbirth, diarrhea, haemoptysis and leucorrhoea. melancholia, conjunctivitis and haemoptysis^[6-7]. Katha (resin) is used as oral contraceptive, chemopreventive, as the best substitute for gum arabic, in the preparations of paints and varnishes, algicidal and antibacterial agent, acrid, bitter, thermogenic, digestive, appetiser, aphrodisiac, vulnerary, anthelmintic, depurative, tonic, used in laryngopathy, flatulence, ulcers, wounds, leprosy, skin diseases, urine incontinence, colporrhagia, toothache, loss of voice, also in cases of mercurial salivation, hoarseness, relaxed sore throat, bleeding, ulcerations and sponginess of gums, bed

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sores, gonorrhoea, otitis, otorrhoea [8-9]. The heartwood is inhaled to check bleeding from nose. Stem is bitter, astringent, acrid, cooling, depurative, anthelmintic, antiseptic, antidyenteric, antipyretic, appetizer, haemostatic and tonic, catarrh, cough, pruritus, leprosy, leucoderma, skin diseases, helminthiasis, anorexia, diarrhoea, dysentery, vitiligo, foul ulcers and wounds, hyperglycaemic, anti-inflammatory, hypotensive, haemoptysis, haematemesis, haemorrhages, fever, anaemia, diabetes, pharyngodynia, and used to improve paper properties, as firewood, for furniture and tools, in dyeing, leather tanning, preservative for fishing nets, viscosity regulator for oil drilling [10].

Various biological activities had evaluated on *A. catechu* Willd. plant such as antipyretic, antidiarrhoeal, hypoglycemic, hepatoprotective, antileprotic, antimicrobia, antibacterial, antifertility, immunomodulatory, hypotensive. From the extensive literature survey, it had noticed that *A. catechu* Willd. plant roots seems to be a potential part for thorough investigation, therefore, the present study was carried out to standardize the roots using chemical, and biological means so that this might be an important ingredient for ulcer remedies [11-14].

2. Material and methods

2.1 Plant material

Roots of *Acacia catechu* Willd. collected from the beat of Trilokpur block, Village Bhood under the range of Raipur Rani, Panchkula, after having permission of Forest Department of Raipur Rani, Panchkula. Dr. H.B. Singh (Scientist and Head, Raw materials Herbarium and museum, NISCAIR, Delhi) identified the plant material under a voucher specimen no -NISCAIR/RHMD/CONSULT/-2009-10/1278/82.

2.2 Solvents and Chemicals

Indomethacin, lansoprazole & ranitidine obtained as a gift sample from Ranbaxy Pvt. Ltd., Gurgaon. Aspirin and sodium carboxy methyl cellulose purchased from RANKEM, RFCL, New Delhi.

2.3 In-vivo antiulcer activity

2.3.1 Animals

Wistar albino rats of either sex (150-175 g) procured from Institutional Animal house, Hindu College of Pharmacy, Sonapat. Throughout the experimental period, the animals housed in cages; provided with food (pellet diet) and water *ad libitum*. Animals fasted 24 h prior to experiment with free access to drinking water. The animals maintained at a temperature range of 22–25 °C. Study had conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee of Hindu College of Pharmacy, Sonapat.

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2.3.2 Acute toxicity studies

The animals were fasted for 24 h before experimentation with free access to drinking water. A total of twenty animals were used divided into four groups consisting of five animals each; which received a single oral dose (2000 mg/kg body weight) of ethanol and aq. extracts of *Acacia catechu* Willd. Animals were observed individually during first 30 min. after dosing, periodically during the first 24 h (With special attention during first 4 h) and thereafter one time daily for a period of 14 days for major changes, changes in behavior and mortality, if any [15].

2.3.3 Selection of dose

The acute toxicity study showed that the ethanolic and aqueous extracts of roots of *Acacia catechu* Willd. found to be safe up to the dose of 2000 mg/kg body weight. Therefore 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of the safe dose selected for experimental purpose. The dose of standard drug selected as per literature survey.

2.3.4 Aspirin + Pylorus ligation- induced ulcer model:

The animals were fasted for 24 h before experimentation with free access to drinking water. The animals divided into six groups consisting of six each. The test drug, aspirin and standard antiulcer drug prepared in 0.5% sodium carboxy methyl cellulose (CMC) suspension as vehicle and administered orally daily (10ml/kg body weight). Group I received standard dose (Famotidine 20mg/kg body weight) orally for 7 days. Group II received aspirin alone (200 mg/kg p.o.). Group III and group IV received total ethanolic extract of roots of *Acacia catechu* Willd. at a dose of 200 & 400 mg/kg body weight, respectively for 7 days administered orally. Group V and group VI received aqueous extract of roots of *Acacia catechu* Willd. at a dose of 200 & 400 mg/kg body weight respectively for 7 days administered orally. From days 5-7, all animals of each group received aspirin orally as an aq. suspension at a dose of 200 mg/kg, two hours after the administration of respective drug treatment. Animals in all groups fasted for 18 h after the respective assigned treatment. The abdomen opened by a small midline incision below the xiphoid process and pylorus portion of stomach lifted out and ligated under anesthesia (anesthetic ether). The stomach sutured with interrupted sutures. Four hours after pylorus ligation, the rats sacrificed and stomach had removed. The gastric contents collected, centrifuged and volume of the supernatant had expressed as ml/100g body weight. Free and total acidity had been determined by titrating with 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicator. The stomach incised along the greater curvature and observed for ulcers. The no. of ulcers counted using a magnifying glass.

2.3.5 Absolute alcohol-induced ulcer model [16-17]

The animals fasted for 24 h before experimentation with free access to drinking water. The animals divided into six groups consisting of six each. The test drug and standard antiulcer drug prepared in 0.5% sodium carboxy methyl cellulose (CMC) suspension as vehicle and administered orally daily (10 ml/kg body weight).

Group I received standard dose (Lansoprazole 20 mg/kg body weight) orally. Group II received alcohol alone (1 ml of absolute alcohol p.o.). Group III and group IV received total ethanolic extract of roots of *Acacia catechu* Willd. at a dose of 200 & 400 mg/kg body weight respectively. Group V and group VI received aqueous extract of roots of *Acacia catechu* Willd. at a dose of 200 & 400 mg/kg body weight respectively. The test drug and Lansoprazole administered orally 30 min. before the oral administration of 1 ml of absolute alcohol. Sixty minutes after the last dose, the animals sacrificed and their stomachs and gastric contents had taken. Stomachs removed and kept immersed in 10% formalin for 5 min. Each stomach incised along with greater curvature and examined for linear haemorrhagic lesions in the glandular region. The number & quantity of ulcer induced calculated. Ulcer index (UI) for each stomach calculated and stomachs had again immersed into 10% formalin for 24 h and histopathological

examinations carried out and later the slides photographed. (Table 4.1) (Graph 4.1).

2.3.6 NSAIDs-induced ulcer model [18-19]

The animals were fasted for 24 h before experimentation with free access to drinking water. The animals were divided into ten groups consisting of six each. The test drug and standard antiulcer drug were prepared in 0.5% sodium carboxy methyl cellulose (CMC) suspension as vehicle and administered orally daily (10ml/kg body weight).

Group I received standard dose (Ranitidine 150 mg/kg body weight) orally. Group II received 1 ml of vehicle orally. Group III and group IV received total ethanolic extract of roots of *Acacia catechu* Willd. at a dose of 200 & 400 mg/kg body weight respectively. Group V and group VI received aqueous extract of roots of *Acacia catechu* Willd. at a dose of 200 & 400 mg/kg body weight respectively. All the treatments were administered orally. One hour after treatment, all the animals received indomethacin (100 mg/kg body weight) administered orally to induce gastric ulcer. Four hours after treatment with indomethacin, the animals were sacrificed. The stomachs were removed and kept immersed in 10% formalin for 5 min. Each stomach was incised along with greater curvature and examined for linear haemorrhagic lesions in the glandular

region. The number & quantity of ulcer induced was calculated. Ulcer index (UI) for each stomach was calculated. Stomachs were again immersed into 10% formalin for 24 h and histopathological examinations were carried out and later the slides were photographed. (Table 4.2) (Graph 4.2).

2.4 Statistical Analysis

All *In-vivo* experiments result had expressed as Mean±SEM; n= 6 in each group *P<0.01 for each value. Evaluation of data was done by Dunnett comparison test following one way ANOVA with the level of significance set at P < 0.01. All the *in-vitro* experimental results were expressed as mean ± S.D. following Dunnett comparison test with standard using one way ANOVA P values >0.05 were considered not significant.

3. Results

3.1. *In-vivo* antiulcer activity

3.1.1 Acute toxicity studies

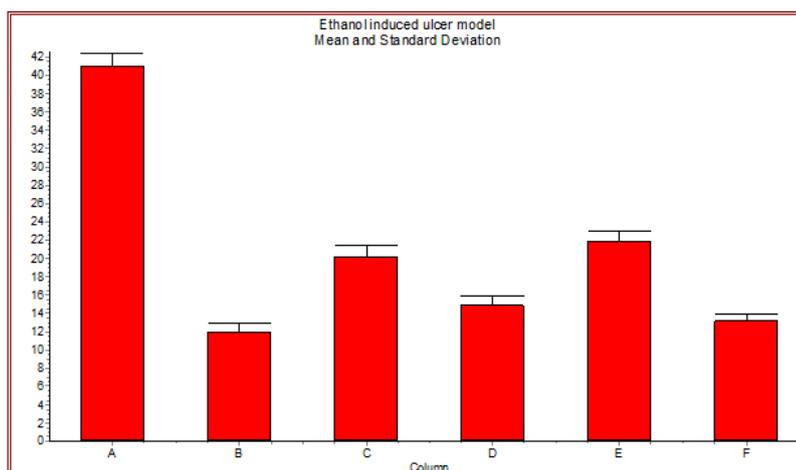
Ethanolic and aqueous extracts of *Acacia catechu* Willd. showed no acute toxicity at fixed dose level (2000 mg/kg b. wt.).

3.1.2 Absolute alcohol-induced ulcer model

Table 1: Results of antiulcer activity of ethanol induced ulcer model

Treatment (p.o.)	Dose (mg/kg b. wt.)	Lesion area(mm ²)	Ulcer Index	% Inhibition
Control	0.908 ± 0.0006	41.00 ± 0.58*
Standard	30 mg	0.050 ± 0.0005	12.00 ± 0.37*	70.73
<i>A. catechu</i> Total Ethanolic extract	200 mg	0.559 ± 0.0006	20.16 ± 0.48*	50.85
	400 mg	0.191 ± 0.0004	14.83 ± 0.40*	63.83
<i>A. catechu</i> Aq. Extract	200 mg	0.487 ± 0.0006	21.83 ± 0.48*	46.76
	400 mg	0.042 ± 0.0006	13.17 ± 0.30*	67.87

#Data are expressed as Mean±SEM; n= 6 in each group *P<0.01 for each value. Evaluation of data was done by Dunnett comparison test with control following one way ANOVA



- A- Control
- B- Lansoprazole 30 mg/kg b. wt.
- C- *A. catechu* total ethanolic extract 200mg/kg b.wt.
- D- *A. catechu* total ethanolic extract 400mg/kg b.wt.
- E- *A. catechu* aq. extract

Where X-axis represents various extract's dose and Y-axis represents ulcer index mean and standard deviation respectively.

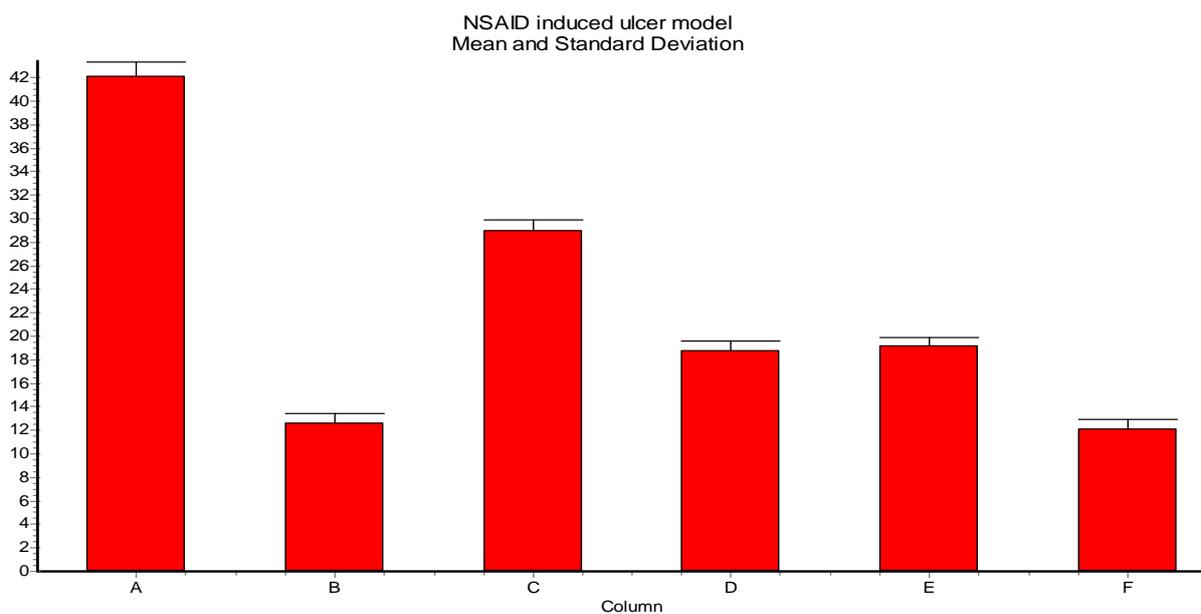
Graph 1: Histogram of absolute alcohol Induced Ulcer Model

3.1.3 NSAIDs-induced ulcer model

Table 2: Results of antiulcer activity by NSAIDs induced ulcer model

Treatment (p.o.)	Dose (mg/kg b. wt.)	Lesion area (mm ²)	Ulcer Index	% Inhibition
Control	1.364 ± 0.0007	42.17 ± 0.47*
Standard	30 mg	0.110 ± 0.0004	12.67 ± 0.33*	69.95
<i>A. catechu</i> Total Ethanolic extract	200 mg	0.372 ± 0.0006	29.00 ± 0.33*	31.33
	400 mg	0.145 ± 0.0006	18.83 ± 0.30*	55.35
<i>A. catechu</i> Aq. Extract	200 mg	0.320 ± 0.0006	19.17 ± 0.30*	54.54
	400 mg	0.089 ± 0.0006	12.17 ± 0.30*	71.14

#Data are expressed as Mean ± SEM; n= 6 in each group *P<0.01 for each value. Evaluation of data was done by Dunnett comparison test with control following One way ANOVA



Graph 2: Histogram of NSAID Induced Ulcer Model

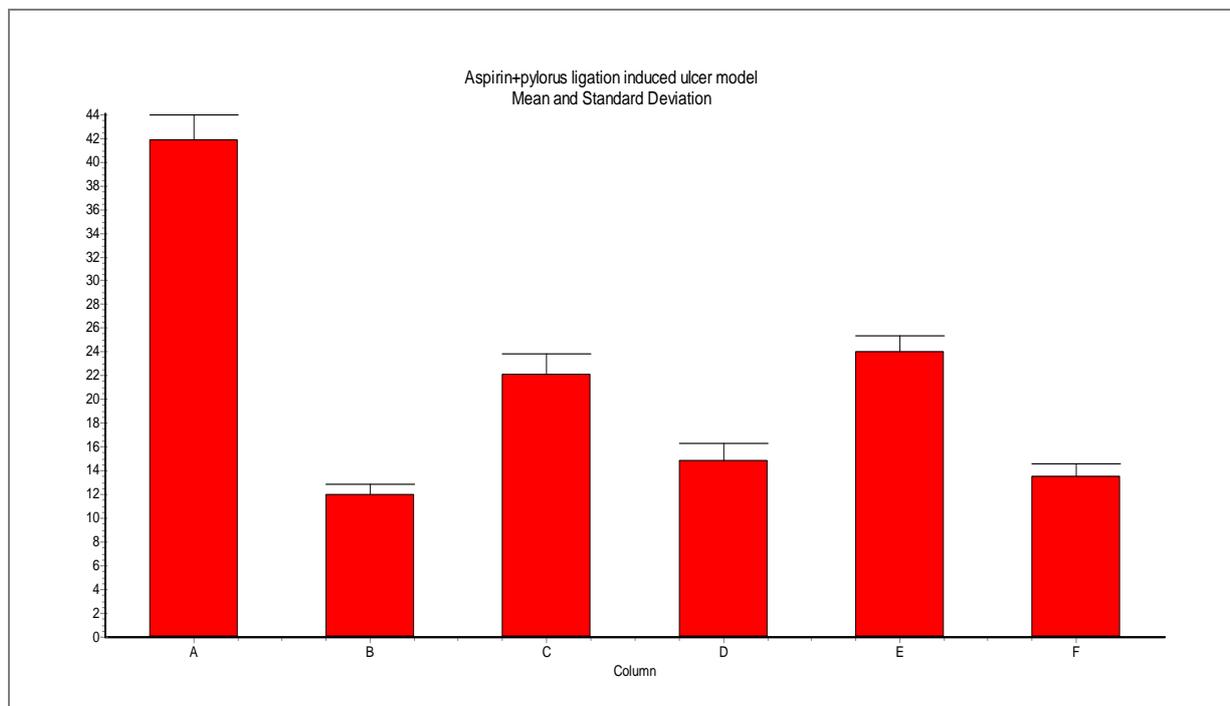
Where X-axis represents various extract's dose and Y-axis represents ulcer index mean and standard deviation respectively.

3.1.4 Aspirin + Pylorus ligation-induced ulcer model

Table 3: Results of antiulcer activity by NSAIDs induced ulcer model

Treatment (p.o.)	Dose (mg/kg b. wt.)	Lesion area (mm ²)	Ulcer Index	% Inhibition
Control	1.364 ± 0.0007	42.17 ± 0.47*
Standard	30 mg	0.110 ± 0.0004	12.67 ± 0.33*	69.95
<i>A. catechu</i> Total Ethanolic extract	200 mg	0.372 ± 0.0006	29.00 ± 0.33*	31.33
	400 mg	0.145 ± 0.0006	18.83 ± 0.30*	55.35
<i>A. catechu</i> Aq. Extract	200 mg	0.320 ± 0.0006	19.17 ± 0.30*	54.54
	400 mg	0.089 ± 0.0006	12.17 ± 0.30*	71.14

#Data are expressed as Mean ± SEM; n= 6 in each group *P<0.01 for each value. Evaluation of data was done by Dunnett comparison test with control following One way ANOVA



Graph 3: Histogram of Aspirin + Pylorus ligation induced ulcer model

4. Discussion

Acacia catechu Willd is thorny trees and distributed throughout the India. These are widely planted in the tropical and subtropical regions ascending upto an altitude of 1200-1500 m. Morphological and microscopical studies of the plant were carried out to identify the plant characteristics of a specific part and to establish the pharmacognostic standards for the plant. For the evaluation of antiulcer activity, aspirin+pylorus ligated ulcer model, ethanol induced ulcer model and NSAID's induced ulcer mode were used. Ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcers. NSAIDs such as indomethacin have the ability to cause gastroduodenal ulceration and this effect is related to the ability of these agents to suppress prostaglandin synthesis. In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucus blood flow, and regulating mucosal cell turnover and repair. Thus, the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration. *Acacia catechu* aqueous extract ($P < 0.01$) reduced the ulcer index and afforded significant protection against ethanol-induced ulcer among the four extracts (ACTE, ACAq., AATE, AAAq.) used for evaluation in present study. *Acacia catechu* Willd. aqueous extract ($P < 0.01$) showed maximum percentage inhibition (71.14) of ulcer among all four extracts (ACTE, ACAq., AATE, AAAq.).

5. Conclusion

Considering several existing theories on peptic ulcer and number of preclinical reports mentioned in the review, we could conclude that studies with new active principles

obtained from plant sources can result in novel and effective pattern of treatment. The results of this study show that aqueous extract, display antiulcer activity, as evidenced by their significant inhibition of the formation of ulcers induced by different models. However, further pharmacological and toxicological investigation is required, to delineate the mechanism of action and their toxic effects

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