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Gastroprotective effect of *Acacia nilotica* young seedless pod extract: Role of polyphenolic constituents

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ABSTRACT

Objective: To systematically evaluate antiulcer potential of *Acacia nilotica* in different ulcer models in rats. **Methods:** Different extracts [ethanolic, 50% hydroethanolic (50:50), 70% hydroethanolic (70:30) and aqueous] of young seedless pods were examined in pylorus ligation induced gastric ulcers in rats. Various parameters like, volume of gastric acid secretion, pH, free acidity, total acidity, ulcer index , mucin content and antioxidant studies were determined and were compared between extract treated, standard and vehicle control following ulcer induction. The most active extract was also evaluated in swimming stress induced and NSAID induced gastric ulceration. **Results:** Among different extracts of young seedless pods only hydroethanolic extracts showed significant antiulcer activity in pyloric ligation induced ulceration. Even more the 70% hydroethanolic extract showed better protection as compared to 50% hydroethanolic extract. Further 70 % hydroethanolic extract also showed significant mucoprotection in swimming stress induced and nonsteroidal antiinflammatory drugs induced gastric ulceration. **Conclusions:** The results of present study concluded that the hydroethanolic extract of young seedless pods of *Acacia nilotica* has antiulcer activity in pylorus ligation, swimming stress and NSAID induced rat ulcer models. The extract containing more amount of phenolic components show high antiulcer activity, indicating the phenolic component of the extract to be responsible for the activity of the extracts.

1. Introduction

Peptic ulcer is a heterogeneous disease that is a major health hazard both in terms of morbidity and mortality. It occurs due to imbalance between offensive versus defensive factors. It manifests as break in the gastrointestinal lining bathed by acid and/or pepsin. Various classes of synthetic antiulcer drugs have been used for its treatment like, H_2-blockers, M_1-blockers *etc*, are associated with danger of drug interaction, adverse effects and increased incidence of relapses during ulcer therapy[1]. Therefore, search for an ideal antiulcer drug continues and has also been extended to herbal drugs for their easy availability, better protection, low cost and lesser toxicity.

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Acacia nilotica Subsp *Indica* is a tropical and subtropical tree belonging to family leguminosae- Mimosoideae and distributed throughout the greater part of India, Ceylon, Baluchistan, Egypt, tropical Africa and Natal[2]. *Acacia nilotica* is widely used in various ayurvedic formulations and its parts like bark, leaves pods and flowers have traditionally been proved for various ailments like cancer, cold, congestion, cough, diarrhea, dysentery, fever, hypertension, hemorrhoid, ophthalmic, sclerosis, small pox, tuberculosis, leprosy, bleeding piles, leucoderma and menstrual problems[3]. Phytochemically tannins 25%-60%, mucilage 20%-30%, flavonoids, resins, saponins & alkaloids have been isolated from different parts of *Acacia nilotica*. Pods & leaves of *Acacia nilotica* contain 8% digestible protein (12.4% crude protein) and young seedless pods contain 18%-27% of tannins[4-7]. Moreover young seedless pods of *Acacia nilotica* contain flavonoids, minerals and higher quantity of tannins[8]. Previous pharmacological reports have shown that flavonoids and tannins have

potent gastroprotective effect[9]. It was hypothesized that the young seedless pods of *Acacia nilotica* might possess gastroprotective effect due to presence of flavonoids and tannins. The present study was undertaken to investigate the gastroprotective effect of the different extract of the seedless pods of *Acacia nilotica* using pylorus ligation, swimming stress and indomethacin induced ulcer model.

2. Materials and methods

2.1. Plant material and preparation of extracts

Aerial parts of *Acacia nilotica* were collected from nearby areas of Barnala, Punjab. The botanical identity of the plant material was verified by Dr. H. B. Singh, Head, Department of Botany, NISCAIR, New Delhi, India. Various solvents *viz.* ethanol and ethanol: water (70:30 and 50:50) and water were used for preparation of extract of young seedless pods of *Acacia nilotica* using a percolator. The extracts were dried under reduced pressure using a rotavapor and the remaining viscous mass was freeze dried using a freeze drier.

2.2. Phytochemical screening

All the extracts were screened for different classes of phytoconstituents using specific standard reagents[10].

2.3. Determination of aflatoxins, heavy metals and pesticides

Estimation of aflatoxins, heavy metals, arsenic and pesticides content in *Acacia nilotica* aerial parts was done from Oscar Analytical Pvt. Ltd., Baddi (Solan).

2.4. Quantification of total phenols and flavonoids

Quantification of phenols was based on the standard curve of gallic acid ($y = 0.0147x - 0.0465$; $R^2 = 0.9926$). Briefly, 10 mg of gallic acid was dissolved in 100 mL of 50% methanol (100 μg/mL) and then further diluted to 6.25, 12.5, 25 or 50 μg/mL. One mL aliquot of each dilution was taken in a test tube and diluted with 10 mL of distilled water. Then, 1.5 mL Folin Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min. Four mL of 20% w/w Na_2CO_3 was added in each test tube, adjusted with distilled water up to the mark of 25 mL, agitated and left to stand for 30 min at room temperature. Absorbance of the standard was measured at 765 nm using UV/VIS spectrophotometer against blank, *i.e.*, distilled water. For quantification, 1 g of each extract was macerated (3×2 h) with 15 mL methanol (50%), filtered, filtrates pooled, and volume was made up to 100 mL with methanol (50%) in a volumetric flask. One mL aliquot of the sample was taken in a test tube and diluted with 10 mL of distilled water. Further similar procedure was adopted and absorbance of was measured at 765 nm using UV/VIS spectrophotometer against blank, *i.e.*, distilled water.

Quantification of flavonoids was based on the standard curve of rutin ($y = 0.0029x + 0.0022$; $R^2 = 0.9998$). Briefly, 10 mg of rutin was dissolved in 100 mL of 80% methanol (100 μg/mL), and further diluted to 10, 20, 40, 80 or 160 μg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of methanol (95%), 0.1 mL of aluminium chloride (10%), 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with UV/VIS spectrophotometer. The amount of aluminium chloride (10%) was substituted by the same amount of distilled water in blank.

For quantification, about 1 g of each hydroalcoholic extract was dissolved separately in 25 mL of methanol (80%). Similarly, 0.5 mL of the extract solution was reacted with aluminium chloride for determination of flavonoid content as described above. Parallel determinations were recorded. The amount of aluminium chloride (10%) was substituted by the same amount of distilled water in blank.

2.5. Pharmacological investigations

2.5.1. Animals

Albino wistar rats weighing 150-200 g of either sex, bred at Central Animal House, S. D. College of Pharmacy, Barnala, were divided into 27 groups (n=5) and provided standard pelleted diet (Ashirwad Industries, Chandigarh) & water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee of S. D. College of Pharmacy, Barnala, before carrying out biological studies all procedures were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India.

2.5.2. Pylorus ligation induced gastric ulceration in rats

Overnight fasted rats were anaesthetized with ether. Surgical incision was given in abdomen below the sternum. Stomach was exposed and thread was tied around the pyloric sphincter and a tight knot was applied in such a manner that blood vessels were spared. The abdomen wall was closed by putting sutures. Colloidion was applied over the wound. Cimetidine (10 mg/kg, *p.o.*) was given 15 min prior to surgery as a standard. After 4 hr, animals were sacrificed by decapitation. Abdomen was opened and tied the esophageal end of the stomach. Cut was given and the entire stomach was removed from body of the animal. A small cut was given to the pyloric region just above the knot. Gastric contents were collected in graduated centrifuge tube and were centrifuged at 1 000 rpm for 10 min. Volume of supernatant was noted as volume of acid secreted. Thereafter, 1 mL of supernatant was pipetted out and it was diluted up to 10 mL with distilled water. The pH of this solution was noted with

the help of pH meter. thereafter the solution was titrated against 0.01 N NaOH solution using Topfer's reagent as indicator, which is dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid & total acidity in gastric fluids. At the end point, the solution turns to orange color. The volume of NaOH consumed noted which corresponds to the free acidity. Titration was carried out further till the solution regains pink color. Again the total volume of NaOH will be noted which corresponds to the total acidity. Acidity (mEq/L/100g) will be expressed.

$$\text{Acidity} = \frac{\text{Vol of NaOH} \times \text{Normality} \times 100}{0.1}$$

To calculate ulcer index, stomach was opened along the greater curvature and washed slowly under running tap water. Then, it was put on glass slide and observed under 10× magnification and scored as: 0= Normal colored stomach, 0.5= Red coloration, 1= Spot ulcers, 1.5= Haemorrhagic streaks, 2= Ulcers $\geqslant$ 3 but $\leqslant$5, 3= Ulcers > 5, 4= ulcers with bleeding. Mean ulcer score for each animal was expressed as ulcer index.

Gastric mucin content was determined using alcian blue dye method. Briefly the stomach was removed after scarifying the animals, the glandular portions was excised and opened down the lesser curvature. The everted stomachs were soaked for 2 hours in 0.1% alcian blue 8 GX dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate adjusted to pH 5.8 with HCl. Uncomplexed dye was removed by two successive washes of 15 and 45 min in 0.25 M sucrose. Dye complexed with mucus was diluted by immersion in 10 mL aliquots of 0.5 M $MgCl_2$ for 2 hr. The resulting blue solution was shaken briefly with equal volumes of diethyl ether and the optical density of the aqueous phase measured at 605 nm. The barrier mucus was expressed in terms of μg of alcian blue dye/g of wet stomach glandular tissue[11].

2.5.3. Swimming stress induced ulcers

Wistar rats fasted for 24-36 hours were forced to swim inside the vertical cylinders (height 30 cm, diameter 15 cm) containing water up to 15 cm height, maintained at 23℃. Three hours after the stress, they were removed from the cylinders and sacrificed by a blow on the head. The ulcer index and mucin content was determined as described above. In this test diazepam served as standard (2 mg/kg; *p.o.*). Test drugs were administered 30 min prior to stress[11].

2.5.4. NSAID induced gastric ulcers

Gastric ulceration in rats was induced by drugs and the ability of several agents to either protect against or aggravate this ulceration was observed. The compounds under investigation were administered 30 min to 1 h before indomethacin (20 mg/kg, *p.o.*) administration. The animals were sacrificed after 4 h and the stomachs were examined for the presence of mucosal lesions. The ulcer index and mucin content was determined as described above[11].

2.5.5. Statistics analysis

All data were expressed mean±SEM. For comparison amongst groups *post hoc* one-way ANOVA was performed. *P* value less than 5% ($P<0.05$) was considered to be statistically significant.

3. Results

3.1. Extraction

The yield of ethanolic, 50% hydroethanolic (50:50), 70% hydroethanolic (70:30) and aqueous extracts from the young seedless pods of *Acacia nilotica* Linn was found to be 10.5%, 16.0%, 22.0% and 6.0% (w/w), respectively.

3.2. Phytochemical screening

Results of preliminary phytochemical screening have been shown in Table 1.

3.3. Determination of aflatoxins, heavy metals, pesticides and microbial content

All the extracts showed absence of aflatoxins (B1, B2, G1 and G2), arsenic, pesticides (heptane lindane, heptachlor, aldrin, di-aldrin, HCH, isomer endrin and DDT) and significant microbial content.

3.4. Quantification of total phenols and flavonoids

The total phenol content of ethanolic, 50% hydroethanolic (50:50), 70% hydroethanolic (70:30) and aqueous extracts from the young seedless pods of *Acacia nilotica* Linn was found to be (9.30±0.18)% (w/w), (10.00±0.60)% (w/w), (17.00±0.60)% (w/w) and (4.20±0.27)% (w/w), whereas total flavonoid content was found to be (2.30±0.62)% (w/w), (3.00±0.43)% (w/w), (4.20±0.27)%(w/w) and (1.20±0.37)% (w/w), respectively.

3.5. Effect of different extracts on pylorus ligation-induced gastric ulceration

Significant ($P<0.05$) change in observed parameters *i.e.* volume of gastric secretion, free acidity, total acidity, ulcer index and mucin content was observed in vehicle control group as compared to sham control, indicating significant ulceration, due to accumulation of gastric contents in ligated animals. Treatment with 70% hydroethanolic extract (100 and 200 mg/kg) significantly ($P<0.05$) reduced gastric volume, free acidity, total acidity, ulcer index and increased the

mucin content. Treatment with the same extract at 200 mg/kg significantly (P<0.05) reduced the gastric pH. Similar protection was also observed after treatment with 50% hydroethanolic extract. However the 70% hydroethanolic extract showed better protection as compared to 50% hydroethanolic extract, which was comparable to cimetidine treatment whereas, ethanolic and aqueous extracts were not found to be effective in reducing pylorus ligation-induced gastric ulceration (Table 2).

3.6. Swimming stress induced ulcers

In stress induced ulcer test significant ulceration was observed in vehicle control group, indicated by ulcer index and decreased mucin content. Treatment with the 70% hydroethanolic extract at 100 and 200 mg/kg significantly (P<0.05) decreased the ulcer index and increased the mucin content. Similar protection was also observed after treatment with diazepam (Table 3).

3.7. NSAID induced gastric ulcers

Administration of indomethacin resulted in a significant ulceration in vehicle control group indicated by increased ulcer index up to 5.12±0.13 and decreased mucin content of 28.8±0.77. Treatment with 70% hydroethanolic extract at 100 and 200 mg/kg significantly (P<0.05) decreased the ulcer index and increased the mucin content (Table 4).

Table 1

Chemical groups identified in the different extracts from the young seedless pods of *Acacia nilotica* Linn.

Class of phytoconstituents	Ethanolic	Ethanol: water (50:50)	Ethanol: water (70:30)	Water
Extract				
Alkaloids	+	+	+	+
Anthraquinone glycosides	–	–	–	–
Cyanogenic glycosides	–	–	–	–
Cardiac glycosides	–	–	–	–
Steroids/Triterpenoids	–/–	–/–	–/–	–/–
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Coumarins	–	–	–	–
Tannins	+	+	+	+
Carbohydrates	+	+	+	+
Proteins	–	–	+	+

+: present, – : absent.

Table 2

Effect of different extracts from young seedless pods of *Acacia nilotica* on pylorus ligation induced gastric ulceration in rats (n=5).

Group name	Volumeof gastric secretion(mL/100g)	pH	Free acidity (meq/L/100g)	Total acidity (meq/L/100g)	Ulcer index	Mucin content (μ g of alcian blue dye per g of wet glandular tissue)
Sham control	–	–	–	–	–	43.20±1.03
Vehicle Control	5.18±0.07*	2.38±0.06	66.12±0.06*	197.20±0.34*	5.26±0.05*	25.00±0.06*
EE 50 mg/kg	4.40±0.03	2.58±0.04	62.00±0.04	192.20±0.44	5.02±0.07	24.20±0.68
EE 100 mg/kg	4.16±0.04	2.64±0.05	58.92±0.06	188.80±0.34	4.92±0.09	30.40±0.46
EE 200 mg/kg	3.90±0.04	2.40±0.04	58.12±0.04	184.60±0.36	4.60±0.07	30.80±0.46
EWA 50 mg/kg	4.28±0.03	2.46±0.05	58.10±0.03	174.60±0.46	5.14±0.05	28.20±0.77
EWA 100 mg/kg	2.52±0.05[a]	3.46±0.02	44.18±0.03[a]	143.80±0.34[a]	2.12±0.03[a]	34.00±0.90[a]
EWA 200 mg/kg	2.71±0.06[a]	3.74±0.02[a]	43.42±0.03[a]	148.20±0.53[a]	2.86±0.02[a]	36.80±0.95[a]
EWB 50 mg/kg	4.60±0.06	3.12±0.02	58.44±0.04	178.60±0.38	4.60±0.03	27.20±0.84
EWB 100 mg/kg	4.40±0.06	3.08±0.03	54.16±0.05	162.80±0.44	4.42±0.07	31.40±0.83[a]
EWB 200 mg/kg	3.80±0.06	3.24±0.04	50.18±0.05[a]	158.00±0.46[a]	3.94±0.06	31.80±0.72[a]
AQE 50 mg/kg	4.03±0.04	2.46±0.03	64.22±0.04	196.80±0.38	5.34±0.04	26.60±0.66
AQE 100 mg/kg	3.46±0.05	2.52±0.03	62.68±0.03	194.60±0.46	5.08±0.03	26.40±0.83
AQE 200 mg/kg	3.38±0.04	2.60±0.04	59.00±0.04	186.80±0.52	4.08±0.04	28.20±0.84
CMD 10 mg/kg	1.66±0.05[a]	5.40±0.03[a]	31.12±0.07[a]	94.20±0.66[a]	1.56±0.06[a]	39.00±0.63[a]

*P<0.05 *vs*. sham control; [a]P<0.05 *vs*. control. CMD: Cimetidine, EE: Ethanolic extract, EWA: 70:30 Ethanolic extract, EWB: 50:50 Ethanolic extract, AQE: Aqueous extract.

Table 3
Effect of EWA of young seedless pods of *Acacia nilotica* on swimming stress induced gastric ulceration model (n=5).

Group name	Sham control	Vehicle control	EWA 50	EWA 100	EWA 200	DZP
Ulcer index	–	3.94±0.05	2.86±0.04	1.90±0.03[a]	1.82±0.06[a]	1.45±0.05[a]
Mucin content (μ g of alcian blue dye per g of wet glandular tissue)	43.20±1.03	27.40±0.83	28.80±0.02	36.40±0.78[a]	37.50±0.06[a]	39.40±0.63[a]

[a]P<0.05 *vs.* control. EWA: Ethanol water (70:30) extract, DZP: Diazepam.

Table 4
Effect of EWA of young seedless pods of *Acacia nilotica* on indomethacin induced anti-ulcer activity model (n=5).

Group name	Sham control	Vehicle control	EWA 50	EWA 100	EWA 200	CMD-10
Ulcer index	–	5.12±0.13	4.92±0.13	3.02±0.03[a]	2.87±0.06[a]	1.10±0.05[a]
Mucin content (μ g of alcian blue dye per g of wet glandular tissue)	43.20±1.03	28.80±0.77	30.10±0.53	37.60±0.92[a]	38.10±0.75[a]	39.20±0.61[a]

[*] [a]P<0.05 *vs.* control. EWA: Ethanol:water (70:30) Extract; CMD-10: Cimetidine.

4. Discussion

Physiological stressors are known to produce gastric ulcers by complex central and peripheral mechanism[12]. The present study investigated the effect of aqueous, 50% hydroethanolic (50:50), 70% hydroethanolic (70:30) and ethanolic extracts from the young seedless pods of *Acacia nilotica* Linn on pylorus ligation, swimming stress and indomethacin-induced gastric ulcers. Among all extracts 50% hydroethanolic and 70% hydroethanolic showed significant protection, whereas ethanolic and aqueous extracts were found to be ineffective. However, 70% hydroethanolic showed maximum activity therefore was only used in other animal ulcer models.

Pylorus ligation-induced ulcers are thought to be caused by increased presence of acid and pepsin in the stomach. This model mimics the most common clinical condition of chronic constipation leading to gastritis and ulceration because of low gastric motility which leads to prolonged gastric acid secretion thus increasing offence. Therefore, the attenuation of induction of ulceration by hydroethanolic extract of young seedless pods of *Acacia nilotica* in this model represents its significant antisecretory activity. Further, the essential criteria, which determine the status of mucosal defense barrier against the offensive assault of acid-pepsin is the quality and quantity of gastric mucus secretion. Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms including lessening stomach wall friction during peristalsis and acting as an effective barrier to the back diffusion of hydrogen ions[13]. As significant increases in mucin content has also been observed with 50% and 70% hydroethanolic extracts reduced the pylorus ligation-induced ulceration in a dose-dependent manner suggesting their mucosal barrier strengthening ability in addition to antisecretory activity.

Several physical and psychological factors are involved in the genesis of stress-induced ulcers[14]. Increase in gastric motility, vagal over activity, mast cell degranulation, decreased mucosal blood flow, and decreased prostaglandin synthesis are some of the important factors among them[15,16]. The 70% hydroethanolic extract treatment resulted in protection of stress induced ulcers, indicating its antistress potential. However, further studies in future will provide more information to understand its exact mechanism of antistress effect.

Non-steroidal anti-inflammatory drugs like, aspirin, indomethacin *etc*. are known to induce ulcers during the anti-inflammatory therapy. The same has been implemented in laboratory to induce ulcers in experimental animals[17,18]. These drugs induces ulcers by inhibition of prostaglandin synthesis through the cycloxygenase pathway. Inhibition of prostaglandin production results in increased acid production and decreased cytoprotective mucus formation, which can lead to induce gastrointestinal ulcer[19]. ROS also has an important role in the mucosal damages caused by indomethacin and other agents[18]. SOD, GST and CAT are some of the antioxidant enzymatic defense mechanism. It has been reported that SOD activity in rat stomach tissues is decreased by non-steroidal anti-inflammatory drugs[19,20]. The polyphenolic compounds exert antioxidant property by free radical scavenging and metal chelating properties. Many phenolic antioxidants that are widely distributed in plants have shown free radical scavenging property. The 70% hydroethanolic extract showed marked gastroprotective properties as evidenced by its significant inhibition of the formation of gastric lesions (in terms of length and number) induced by indomethacin.

Preliminary phytochemical screening showed the presence of flavonoids and phenolic components in the active extracts. Flavonoids and phenolic components have been found to possess antiulcer activity. These components causes coating of gastric wound, form complexes with proteins of cell wall, chelate free radicals and reactive oxygen species, stimulate the contraction of wound, increasing the formation of new capillaries and fibroblasts. Tannins causes precipitation of mucosal proteins forming an impervious layer over hence protects the underlying mucosa from injury and irritant component[21]. Phenols stimulate PGE formation based on their action as co substrates for the peroxidase reaction[22]. In quantitative test the 70% hydroethanolic extract showed

highest amount of total phenols and flavonoids content followed by 50% hydroethanolic. These results corresponds the results of *in vivo* studies in which the 70% hydroethanolic showed maximum protection. Hence the antiulcer activity of the extract might be due to the presence of polyphenolic components.

The results of present study concluded that the hydroethanolic extract of young seedless pods of *Acacia nilotica* has antiulcer activity in pylorus ligation, swimming stress and indomethacin induced ulcer rat models. The extract containing more amount of phenolic components show high antiulcer activity, indicating the phenolic component of the extract to be responsible for the activity of the extracts. However, more studies are required to understand the exact mechanism and phenolic components responsible for the activity of the extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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