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Original Article

GASTRIC ANTISECRETORY AND ANTIULCER PROPERTIES OF ETHANOLIC EXTRACTS OF LEAVES OF *ANDROGRAPHYS PANICULATA*

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ABSTRACT

Objective: To study the gastric anti-secretory and cytoprotective action of ethanolic extract of leaves of *Andrographys paniculata*.

Methods: Effect of ethanolic extract of leaves of *A. paniculata* gastro-antisecretory and cytoprotective activity was studied in vivo by using gastric wall mucus determination, hypothermic restraint stress-induced ulcers, idomethacin-induced gastric ulcers, reserpine-induced gastric ulcers and necrotizing agent-induced gastric ulcers (cytoprotection studies) methods.

Results: Ethanolic extract of leaves of *A. paniculata* produced a significant decrease in gastric secretion in pylorous ligated rats and a highly significant cytoprotective effect against 80% ethanol-0.6 M HCl, 0.2 M NaOH, and 25% NaCl-induced cytodestruction.

Conclusion: Pretreatment with the extract significantly prevented hypothermic stress-induced gastric wall mucus depletion. These findings suggest that a significant antisecretory and cytoprotective action *A. paniculata* can be responsible for its antiulcer activity.

Keywords: *A. paniculata*, Gastric secretion, Gastric ulcers, Cytoprotection, Gastric wall mucus.

INTRODUCTION

Andrographis paniculata (Acanthaceae) is an Indian herbal medicine used as an anti-inflammatory and antipyretic drug for the treatment of fever, cold, laryngitis, diarrhea, and rheumatoid arthritis [1]. Experimental studies have revealed numerous pharmacological activities by extracts of *A. paniculata* and its related chemical constituents, such as anti-inflammatory, hepatoprotective, antimalarial, antibacterial, antithrombotic, immune stimulant, antidepressive, antiallergic, central nervous system disorders, anti HIV, and anticancer [2-18]. Diterpenoids and flavonoids are the primary constituents found in leaves of *A. paniculata*, in particular, andrographolide is the major metabolite. Recent reports revealed that andrographolide may be beneficial in the treatment of endotoxic shock by suppressing the production of nitric oxide (NO) and expression of inducible nitric oxide synthase, reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), are important toxic metabolites involved in the intracellular killing of microorganisms and tissue damage by phagocytes during inflammation.

Moreover, stimulated neutrophils are more likely to adhere to extracellular matrix protein, where they become "activated" to release hydrolytic enzymes and large amounts of ROS that results in tissue damage. The other species of the same genera are being used as an antidepressant, anti-ulcer, memory and learning enhancers, etc [19-26]. The various medicated formulas for antiulcer contain *Andrographis* species. But till now there is no scientific work has been reported on its antiulcer activity. Therefore the current study was aimed to explore this indigenous plant for antiulcer activity.

MATERIAL AND METHODS

Extract preparation

Andrographis paniculata were procured from the local market whole plant of *Andrographis paniculata* was collected from botanical herbarium (Herbarium no. Taxonomy/212B);Orissa, India in the months of March 2013. The powdered drug (500g) was extracted using a percolation method and 96% ethanol. The solvent was then removed at low temperature under reduced pressure, and the extracts were stored in a refrigerator for pharmacological studies. The extract yield was 5.6% (w/w) in terms of starting material. The dried extract was dissolved in water before administration to the animals. Wistar albino rats of either sex, approximately of the same age, weighing 150 – 200 g, and fed on standard chow diet were used. They were divided into groups of six animals each. The distribution of animals in groups, the sequence of trials, and the treatments were randomized. The solutions of the ulcerogenic drugs and necrotizing agents were freshly prepared before administration. The animals were killed by ether euthanasia. The stomachs were removed, opened along the greater curvature, washed with saline and examined with a 6.4 x binocular magnifier. Lesions were also assessed by two observers unaware of experimental protocols. Gastric lesions induced by the drugs used in this study were multiple in each stomach. They were evaluated singly according to their dimensions and severity and scored between 0 and 10, no visible ulcers, deep lesion with diameter greater than 8mm, in each stomach. The scores for each single lesion were then totaled. The results refer to average lesion score ± SEM;statistical analysis of the severity of gastric ulcers was done by Student's Mest.

Gastric wall mucus determination

A slightly modified procedure of Corne et al. (1974) was followed. The glandular segments rom stomachs which has been pended along their greater curvatures were removed and weighed, Each segment was transferred immediately to 10 ml of 0.1% w/v Alcian blue (dye) solution. Color absorbance was recorded by means of a spectrophotometer (LKB) set at an optimal wave length of 596 nm. The quantity of Alcian blue extracted per g wet weight of glandular tissue was then calculated from standard curves.

Experimental gastric lesions

Pylorus ligated (Shay) rats:The animals were fasted for 48 h with access to water ad libitum before the pylorus was ligated under light anesthesia, care being taken not to cause bleeding or to occlude blood vessels. *A. paniculata* extract was administered (i.p.) just after pylorus ligation. The animals were killed 6h after the pylorus ligation, the stomachs removed, contents collected and measured. Each stomach was examined for lesions.

Hypothermic restraint stress-induced ulcers

The method Levine (1971) was followed with slight modification. The animals were fasted for 36 h with access t water ad libitum and, 1h after the drug treatment, they were immobilized in restraint

cages and placed in a ventilated refrigerator maintained at a temperature of-4°C for 2 h. The animals were then killed and the stomachs were excised. They were examined for the severity of intraluminal bleeding according to the following arbitrary scale:0-10, no blood detectable;thin blood follows the rugae with blood clots in certain areas;extensive covering of the whole gastric mucosal surface with thick blood. After removing the blood, the lesions in each stomach were scored.

Idomethacin-induced gastric ulcers

Idomethacin was suspended in 1% caboxymethylcellulose in water 6 mg/mL) and administered p.o. at a dose of 30 mg/kg (0.5 mL/100 g) to rats fasted for 36 h. A.galanga extract was administered 30 min before indomethacin. The rats were killed 6 h after indomethacin administration.

Reserpine-induced gastric ulcers

Reserpine (5mg. Kg i.m.) was administered to rats fasted for 36 h. the extract was administered 30 min before the administration of reserpine. The animals were killed 24 h later.

Necrotizing agent-induced gastric ulcers (cytoprotection studies)

The experiments were carried out on make Wistar rats fasted for 36 h with access to drinking water ad libitum. The animals were given 1 mL of either 80% ethanol, 0.6 M HC1, 0.2 M NaOH, or 25% (w/v) NaCl p.o. The extract was administered 30 min before the necrotizing agents, and the animals were killed 1 h after the administration of necrotizing agents.

RESULTS AND DISCUSSION

In Shay rats, ligation of pylorus for 6 h produced mild ulcer mainly located in the forestomach (Table 1). Pre-treatment of animals with *A. paniculata* extract significantly decreased gastric secretions and ulcers. The animals subjected to hypothermic restraint stress exhibited intraluminal bleeding, depletion of gastric mucosal wall and ulceration, mainly in the glandular segment of the stomach (Table 2). Treatment of animals with the extract of *A. paniculata* showed significant protective effect on stress-induced changes in gastric mucosa.

The anti-ulcer activity in these models might be attributed to an anti-secretory effect of the extract. In human subjects also, the *A. paniculata* has been reported to relieve the symptoms of heart burn and dyspepsia. The increase in gastric secretion has been considered as a pathogenic mechanism responsible for stress-induced gastric lesions.

The gastric lesions induced by various necrotizing agents including ethanol, HC1, NaOH and NaCl, produced patcheal ulcers of various size, usually parallel to the major axis of the stomach. The intensity of the ulcers was significantly reduced in the animals treated with *A. paniculata* extract (Table 3).

These findings argue for a 'cytoprotective' effect pf *A. paniculata* extract according to the definition of Robert. The necrotizing agents produce ulceration in gastric mucosa by depleting gastric mucus depletion as observed in our study, might be responsible for increasing mucosal resistance against noxious chemicals.

Table 1: Effect of ethanolic extract of *A. paniculata* on the gastric secretion and ulcer index in 6 h pylorus ligated (Shay) rats

Treatment	Dose (mg/ kgl)	Volume of gastric secretion	Ulcer index (mean ±SE)
Control		9.41 ± 1.09	2.0 ± 0.06
A. paniculata	500	6.16 ± 0.41*	0.16 ± 0.00*

Six animals were used in each test group; *p < 0.05 (Student's f-test)

Table 2: Effect of ethanolic extract of *A. paniculata* on gastric wall mucus changes in rats

Groups	Dose (mg/kg p.o.)	Gastric wall mucus (tig Alcian blue/g wet glandular tissue)	Intraluminal bleeding (mean score ± SE)	Ulcer index (mean ±SE)
Normal unstressed		310.74 ± 30.78 = (7)		
Hypothermic stressed		200.72 ± 10.05 (7)	2.50 ± 0.56 (6)	31.2 ± 2.40 (6)
Hypothermic stressed + *A. paniculata*	500	360.04 ± 40.99 (7)	0.66 ± 0.49** (6)	19.8 ± 3.71* (6)

Numbers in parenthesis indicate the number of animals used; **Compared with the stressed (control) group; Six animals were used in each test group; *p < 0.05 (Student's f-test)

Table 3: Effect of ethanolic extract of *A. paniculata* against the gastric lesions induced by various necrotizing agents in rats

	Ulcer Index mean ± SE	
Procedure	Control	*A. paniculata* (500 mg/kg p.o.)
80% Ethanol	7.16 ± 0.3	0.16 ± 0.16*
0.6 M HCl	7.16 ± 0.4	1.50 ± 0.34**
0.2 M NaOH	7.16 ± 0.4	0.33 ± 0.21*
25% NaCl	6.83 ± 0.83	0.33 ± 0.21*

Six animals were used in each test group; *p < 0.001, Student's t-test as compared to respective controls

The gastric mucus coat is thought to be important in both preventing damage and in facilitating the repair of gastric epithelium. However, pre-treatment of animals with *A. paniculata* extract did not protect the gastric mucosa against indomethacin and reserpine (Table 4).

The failure of the extract to protect against gastric ulceration induced by indomethacin clearly excludes a prostaglandin mediated protective mechanism.

The phytoconstituents of the rhizome of *A. paniculata* responsible for anti-ulcer activity are not known.

The seeds of this plant have been reported to contain two highly active components, namely acetoxychavicol and acetoxyeugenol.

Table 4: Effect of ethanolic extract of *A. paniculata* on gastric mucosal damage induced by indomethacin and reserpine

Treatment	Dose (mg/ kg p.o.)	Ulcer index (mean ±SE)	P value (Student's t-test)
Indomethacin		35.66 ± 3.49	
Control			
A. paniculata	500	29.33 ± 3.85	p > 0.05
Reserpine		40.66 ± 1.11	
Control			
A. paniculata		26.83 ± 3.19	p > 0.05
Six animals were used in each group			

CONCLUSION

The effect of *Andrographis paniculata* extract has been studied on experimentally induced gastric ulcers in rats. The ethanolic extract of *A. paniculata* at a dose of 500 mg/kg, significantly reduced the intensity of gastric mucosal damage induced by pyloric ligation and

hypothermic restraint stress in rats. It produced a significant decrease in gastric secretion in pylorous ligated rats and a highly significant cytoprotective effect against 80% ethanol-0.6 M HCl, 0.2 M NaOH, and 25% NaCl-induced cytodestruction. Pretreatment with the extract significantly prevented hypothermic stress-induced gastric wall mucus depletion. These findings suggest that a significant antisecretory and cytoprotective action *A. paniculata* may be responsible for its antiulcer activity.

Ethics committee approval

Animals used in this present study were purchased from the government licensed institute of Andhra Pradesh, JNTU, India, that follows proper procedures regarding animal handling. Doctoral committee of center for post Graduate studies and Research, JNTU, Hyderabad was approved to do this research.

COMPETING INTERESTS

The authors declare that there are no competing interests.

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