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EVALUATION OF GASTRIC ANTIULCER AND ANTIOXIDANT ACTIVITIES IN AQUEOUS EXTRACTS OF *ANNONA SQUAMOSA* AND *ACHYRANTHES ASPERA* IN RATS

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ABSTRACT

Antiulcer activity of aqueous extracts of *Annona squamosa* and *Achyranthes aspera* was studied in Rats by pyloric ligation plus Aspirin induced gastric ulcer method. The antioxidant potential of extracts was studied using *In vitro* Nitric oxide scavenging activity and inhibition of Lipid peroxidation. The ulcer index found to be significantly ($p<0.01$) less in animals treated with 200mg/kg of individual extracts and combination of extracts when compared to vehicle control. The Nitric oxide scavenging activity and lipid peroxidation inhibiting activity is also significant ($p<0.01$) compared to Control.The study revealed the antiulcer activity of aqueous extracts of *Annona squamosa* and *Achyranthes aspera*. The reduction in gastric volume, P^H, acidity, ulcer index supports the antisecretory and antiulcer activity of the extracts. Nitric oxide scavenging activity and lipid peroxidation inhibiting activity of extracts suggests the cytoprotective action, thereby increasing mucosal defense.

KEY WORDS: Gastric antiulcer activity, antioxidant activity, antisecretory, cytoprotection, mucosal defense.

INTRODUCTION

Different classes of drugs including proton pump inhibitors and H_2receptor antagonists are available for the treatment of Gastric ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects, and drug interactions. This has been the rationale for the development of new antiulcer drugs and the search for novel molecule has been extended to herbal drugs that offer better protection and minimize relapses. Gastric ulcer has a multifactorial ethiology which includes excess acid secretion, *Helicobacter pylori*, oxidative stress etc (Schraufstatter *et al*., 1988). *Annona squamosa* and *Achyranthes aspera* are reported to have many medicinal uses. Proved Antiinflammatory (Chavan MJ *et al*., 2009; Gokhale AB *et al*., 2002), antimicrobial (Shivshankar Kanijalal *et al*., 2007; Bhoomika *et al*., 2007) and wound healing properties of both plants has been the driving factor of selecting the plants for antiulcer activity.

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MATERIALS AND METHODS

Experimental animals

The study was conducted on Male Albino rats (Wistar strain) of 150-200g and maintained under standard conditions (room temperature 24-27^{o}C and humidity 60-65%) with 12 h light and dark cycle. The food in the form of dry pellets (Rayan Biotechnologies Pvt Ltd) and water *ad libitum*. Rats were randomly allocated to 5 groups of 6 animals each. The animal experiments were approved by the ethics committee of the institute.

Chemicals and drugs

Ranitidine (Dr.Reddy's,Hyd), sulfanilamide , N-1-naphthylethylenediamine dihydrochloride, Tris-Hcl buffer, potassium chloride, ascorbic acid, ammonium ferrous sulphate, sodium dodecyl sulphate, thiobarbituric acid, glacial acetic acid, butanol, pyridine

Preparation of aqueous extracts

The leaves of *Annona squamosa* and *Achyranthes aspera* were collected locally and identified by department of botany D.N.R.College, Bhimavaram. A voucher specimen was preserved for further reference. They were dried under shade in a room for ten days. Then they were powdered by grinding .The powder was macerated in distilled water for 24 hours. The extract was filtered and air dried to obtain powder (Edwin S *et al*., 2008)**.**

Treatments

For 15 days, Group 1(control) received 0.2 ml of distilled water. Group 2 (Standard) received 50mg/kg Ranitidine. Group 3&4(test1&2) received 200mg/kg *Annona squamosa* extract and *Achyranthes aspera* extract respectively. Group 5 received combination of both extracts.

Aspirin plus Pyloric ligation induced gastric ulcer method

On the 15th day, after 1hr of treatment 200mg/kg of Aspirin was administered orally to all the animals. After half an hour the animals were anesthetized using Ketamine 80mg/kg, abdomen was opened and pylorus was ligated without obstructing blood supply and abdomen was sutured. At the end of 6h the animals were sacrificed and stomach was isolated after ligation of oesophagus (Deshpande SS *et al*., 2003)**.** The gastric juice was collected and volume was measured. Stomach was cut along the greater curvature and ulcer index was evaluated.

Total Acidity

Total acid output of the gastric juice was estimated by titration of 0.1 ml of gastric juice with 0.01 N sodium hydroxide using phenolphthalein as indicator. Acid output was expressed as mEq/L (Hawk PB and Ostor BL, 1965).

ANTIULCER ACTIVITY

Ulcer index

The stomach was fixed in 10% formalin and examined with a lens for ulcers. The ulcer score was given as follows. Normal colored stomach – 0, Spot ulcers- 1, Surface ulcer – 2, Deep ulcer – 3, Perforation – 4 (Ganguly AK and Bhatnagar OP, 1973). The percentage of ulcer inhibition was obtained by following formula:

$$\frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Antioxidant activity

Nitric oxide scavenging activity

The Nitric oxide scavenging activity was measured according to the method of Marcocci et al., 1994. 4ml of different concentrations of test sample, 1ml of Sodium Nitroprusside solution were added in test tubes and incubated at 37^{o}c for 3h. An Aliquot of incubation solution (0.5ml) was taken in a test tube and 0.3ml of Griess reagent was added. The absorbence of chromophore formed was measured immediately at 570 nm. A control was prepared using 0.1 ml of respective vehicle in the place of test sample (Marcocci I *et al*., 1994).

Lipid peroxidation inhibiting activity

Inhibition of lipid peroxidation was determined by the method developed by Okkawa *et al*., 1979. Rat liver tissue weighing 10 gm was homogenized with a polytron homogenizer in ice-cold Tris-Hcl buffer to produce a 25% w/v homogenate. The homogenate was centrifuged at 4000 rpm for 10 min. An aliquot of supernatant 0.1 ml was mixed with 0.1 ml of plant extract of different concentrations, followed by addition of 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06 mM) and 0.1 ml of ammonium ferrous sulphate (0.16mM) and were incubated for 1 hour at 37^{o}C. The reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20 % acetic acid (pH 3.5). The total volume was then made up to 4 ml by adding distilled water and kept in an oil bath at 1000C for 1 hour. After the mixture had been cooled, 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture were added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured at 532 nm. A control was prepared using 0.1ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of the control (Okkawa *et al*., 1979).

Statistical analysis

Statistical analysis was performed using Students t test (Statext 10.0) and significance of difference between treatments was accepted at $p < 0.01$. Data were expressed as mean ± standard error of the mean.

Effect of extracts on Gastric volume, P^H and Acidity

In all treated groups, there was a significant ($p<0.01$) reduction in the gastric volume when compared to vehicle control. The extracts did not exhibit significant decrease in P^H and Acidity.

Effect of extracts on Ulcer index

Aqueous extracts of *Annona squamosa* and *Achyranthes aspera* showed significant ($p<0.01$) reduction in the ulcer index. However the standard drug Ranitidine produced efficient reduction in ulcer index than that of the test groups.

Nitric oxide scavenging activity of extracts

Aqueous extracts of *Annona squamosa* and *Achyranthes aspera* produced significant (p<0.01) lowered levels of nitric oxide and a dose dependent increase was observed when compared to standard. *Achyranthes aspera* extract exhibited efficient reduction of Nitric oxide than that of *Annona squamosa* extract. Standard sample Ascorbic acid proved to be more efficient than the extracts.

Lipid peroxidation inhibiting Activity

Both extracts produced significant (p<0.01) reduction in lipid peroxidation and a dose dependent increase in inhibiting activity compared to standard. However Ascorbic acid proved to be more efficient than extracts.

RESULTS

Table 1. Table shows effect of aqueous extracts of *Annona squamosa* and *Achyranthes aspera* on pyloric ligation plus Aspirin induced Gastric ulcers.

Group	Gastric content (ml)	P^H	Acidity (meq/L)	Mean Ulcer index	% Inhibition of Ulcer
Control	10.58±0.81	1.915±0.30	116.6±8.82	3.2±0.39	0
Standard (50mg/kg)	5.83±0.21*	2.86±0.18**	48.3±5.42*	0.73±0.07*	77.2
Test 1 (200mg/kg)	7.28±0.86*	2.48±0.45***	71.6±8.72*	1.30±0.25*	62.21
Test 2 (200mg/kg)	5.06±1.38*	2.21±0.27	83.3±15.8***	1.11±0.14*	67.73
Test 3(Combination).(200mg/kg)	5.58±0.60*	2.30±0.48**	100±6.32***	1.18±0.19*	65.5

The values are Mean±SEM. Significantly different from control at *p< 0.01, **p< 0.05, ***p< 0.1 (Student t test).Values where *p< 0.01 were considered significant.

Table 2. Table shows *in vitro* Antioxidant Activity of Aqueous Extracts

Sample	Concentration (µg/ml)	Inhibition of nitric oxide radicals (%)	Inhibition of lipid peroxidation (%)
Ascorbic acid	5	48.2±0.26	30.1±0.31
	10	55.2±0.75	42.9±0.15
	25	56.6±0.66	51.6±0.1
	50	64.9±2.05	63.6±0.21
	100	74.8±0.53	80.9±0.1
A.squamosa extract	5	19.3±0.55	13.9±0.2
	10	29.1±0.2	38.6±0.29
	25	39.4±0.63	46.0±0.23
	50	48.2±0.37	61.6±0.06
	100	53.5±0.23	70.8±0.24
A.aspera extract	5	28.4±0.42	20.9±0.13
	10	45.0±2.02	31.1±0.06
	25	52.2±0.1	48.2±0.25
	50	60.2±0.87	62.4±0.4
	100	71.1±0.37	74.6±0.1

The values are Mean±SEM. All Test values are significant different from standard at p< 0.01 (Student t test)

DISCUSSION

Anti ulcer activity:

Peptic ulceration has a multifactorial etiology. The imbalance between the aggressive factors and the protective factors is the major cause. Excessive production of acid, decreased prostaglandin protective action due to drugs and *H.pylori* infection are the major factors worth considering.

In the present study Aspirin plus pyloric ligation method is employed to induce ulcer in adult rats. The present study established potent activity of 200mg/kg aqueous extracts of *Annona squamosa* and *Achyranthes aspera* in significantly (p<0.01) reducing

the Gastric acid secretion and decrease in ulcer index. The effect of extracts is compared with standard drug Ranitidine (50mg/kg). The combination of both extracts exhibited no significant synergistic or additive effect. The exact mechanism of action of extracts is not known but the anti ulcer activity may be attributed to their anti-secretory and mucoprotective action.

Antioxidant activity:

Large quantities of reactive oxygen species are proved to produce tissue damage and aging. Superoxide, hydroxyl radicals, singlet oxygen, Nitrogen species etc are the main cause for oxidative stress. These reactive oxygen species are involved in Lipid peroxidation of the tissue causing damage, immune responses and more importantly the degraded mucosa provides suitable conditions for colonization of *H.pylori*, the main causative factor for peptic ulcer as specified by Marshall and Warren and recent statistics. Endogenous Nitric oxide helps in maintaining the integrity of the tissue. But high amounts of Nitric oxide released during tissue damage causes vasoconstriction and impairment in normal blood flow. Anti oxidants compete for oxygen and reduce the production of nitric oxide. There are restrictions in the use of Synthetic anti oxidants like BHT as they are carcinogenic. Thus as a supportive measure in ulcer treatment Anti oxidants prove to be helpful.

In the present study aqueous extracts of *Annona squamosa* and *Achyranthes aspera* significantly ($p<0.01$) inhibited both Lipid peroxidation and Nitric oxide radical in a dose dependent manner and compared to the standard (Ascorbic acid).

CONCLUSION

Thus the study establish potent antiulcer and antioxidant properties .Reduction in gastric volume and ulcer index reveals anti secretory and anti ulcer potential of extracts and Inhibition of Lipid peroxidation, Nitric oxide radical scavenging activity reveals the gastro cytoprotective efficiency. Further studies are required to monitor anti ulcer activity of extracts in different models of ulcer, stress and investigation of active principles**.**

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