Research Article

Antiulcer and Antioxidant Activity of Leaves of *Andrographys paniculata*

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INTRODUCTION

Gastric ulcers have an effect on thousands of people, and are currently well thought-out a global health problem. It is an imbalance between aggressive and protective factors in the stomach. It affected by various factors like acid–pepsin secretion, mucosal barrier, mucus secretion, blood circulation, cell regeneration, smoking, *H. pylori,* nutritional deficiencies, hereditary predisposition, prostaglandins and epidermal growth factors. Oxygen derived free radicals insinuation, in the pathogenesis of an extensive variety of clinical disorders, anxiety, stress and gastric damage caused by physical, chemical and psychological factors that direct to gastric ulceration in human and animals. Although many products are used for the treatment of gastric ulcers e.g. antacids, anticholinergics, proton pump inhibitors and H_2 receptor antagonists, most of these drugs produce side effect. However, Plant extracts are some of the most attractive sources of new drugs, and have been shown to produce promising results for alternative therapies the treatment of gastric ulcer.

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[1]. 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 nitricoxide 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.[19-29] ROS have been implicated in the etiology and pathophysiology of gastrointestinal inflammation and gastric ulcers, and antioxidant actions have been reported to be effective in the cytoprotection and/or healing in the experimentally induced peptic ulcers. However, until now there is no scientific works reported on its anti-ulcer and antioxidant. Therefore, the present study aimed to explore this indigenous plant for antiulcer and antioxidant activity.

MATERIALS AND METHODS

Drugs and Chemicals

2,2-diphenyl-1-picrylhydrazylhydrate (DPPH), nitroblue tetrazolium chloride (NBT), riboflavin and ascorbic acid were purchased from HiMedia Chemicals Ltd. Rest of the chemicals ($FeSO_4$, $FeCl_2$, MeOH, Deionised water, Sodium Salicylate, etc.) were procured from the departmental chemical stores. All the chemicals and reagents used were of highest commercially available purity. The procurement, housing, diet, etc of animals used for the *in vivo* antioxidant activity is already mentioned. Lansoprazole (100 mg)

tablet, from Ranbaxy Laboratories India used as a reference drug in all the animal models studied. Rests of the chemicals used for the study receive from the institutional chemical house purchasing all chemicals from CDH, Merck pharma, etc.

Preparation of extracts and fractions

Leaves of *Andrographis paniculata* shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in a soxhlet apparatus. The extraction continued until the defatting of the material had taken place. The defatted marc subjected to ethanolic extraction for a period of 6-7 days. The ethanolic extract obtained was dissolved in dist. water and kept overnight to as to settle down the undissolved matter, which filtered off later. The supernatant fractionated with ethyl acetate (400 ml) in separating funnel (250 ml) both fractions dried at 40°C in rotatory evaporator up to a semisolid consistency. Presence of various groups of phytoconstituents has evaluated using different chemical tests.

Administration of the extracts and fractions

Suspensions of ethanolic extract (EA), ethyl acetate (EAC) and ppt. fractions (PPF) were prepared in distilled water using Tween-80 (0.2% v/v) as the suspending agent. Control groups given only the vehicle (0.2% v/v Tween-80 solution) in volume equivalent to that of the plant extracts and fractions.

Screening Models for the Assessment of Antiulcer Activity

Gastric lesions induced by HCl/ethanol the anti-ulcerogenic activity of EA, EAC and PPF derived from *A. paniculata* studied in 150 mM HCl/EtOH induced gastric ulcer with small modifications. Rats have allotted into different groups; each group contained six animals each and fasted for 24 hours prior receiving an oral dose of saline (NaCl 9%, 5 ml/kg), ethanolic extract, ethyl acetate and ppt. fractions (500 (extract), 50 (fractions) mg/kg). Other group received ranitidine (100 mg/kg, p.o) as reference compounds. After 1 hour, all groups orally treated with 1ml of 150 mM HCl/EtOH (40:60, v/v) solution for gastric ulcer induction. Animals were killed 4 hours after the administration of ulcerogenic agent; their stomach were excised and opened along the great curvature, washed and stretched on cork plates. The surface examined for the presence of lesions and the extent of the lesions measured. The number of the lesions along the stomach recorded as ulcer index and percent I was also calculated using the formula.

Stress- induced acute gastric lesion

The antiulcerogenic activity of EA, EAC and PPF derived from *A. paniculata* studied in the hypothermic-restrain stress-induced induced gastric ulcer with small modifications. Rats have allotted into different groups; each group contained six animals each and fasted for 24 hours. After 24 hours of starvation, the animals received single oral dose of ethanolic extract, ethyl acetate and ppt. fractions (500 (extract), 50 (fractions) mg/kg) control group received only vehicle in equal volumes of drug. Other group received ranitidine (100 mg/kg, p.o) as reference compounds. One hour after pretreatment, all groups allowed for the gastric ulceration induction. All animals immobilized inside a closed cylindrical cage maintained at 4°C. After 4 hours the animals were killed. Their stomach excised and opened along the great curvature, washed and stretched on cork plates. The surfaces examined for the presence of lesions and the extent of the lesions measured. The number of the lesions along the stomach recorded as ulcer index and percent inhibition also calculated.

$$\text{Ulcer Index (UI)} = \frac{\text{Number of ulcer in control - Number of ulcer in test}}{\text{Number of animals}}$$

$$\text{Percent Inhibition (\% I)} = \frac{\text{UI control-UI of test}}{\text{UI of control}}$$

In-vitro Antioxidant Activity

O_2-scavenger activity assay

Reaction mixture contained 50 mM PBS (pH 7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg/3ml

NBT, added in that sequence. The reaction started by illumination the reaction the reaction mixture with different concentration of samples. Immediately after illumination, the absorbance measured in spectrophotometer at 590 nm and IC_{50} values were calculated. Ascorbic acid used as positive control. Readings have taken at 15, 30 and 45 min.

Preparation of reaction mixtures & measurement of scavenging
Methodology
100 µl riboflavin solution, 200 µl EDTA solution, 200 µl ethanol and 100 µl of NBT solution was mixed in a borosilicate test tube and the reaction mixture was diluted up to 3 ml with PBS and the whole reaction mixture was illuminated for 15 mins. Just after illumination the reading were taken at 590 nm using PBS as a blank. This has taken as control reading. 100 µl of test solutions i.e. extract and two fractions, 100 µl riboflavin solutions, 200 µl EDTA solution, 200 µl ethanol and 100 µl of NBT solution was mixed in a borosilicate test tube and the reaction mixture was diluted up to 3 ml with PBS and the whole reaction mixture was illuminated for 15 min. Just after illumination the reading were taken at 590 nm using PBS as a blank. This has taken as control reading. Different reaction mixtures were prepared for each test samples.
Percent reduction of O-2 generation calculated as

$$\% \text{ reduction} = \frac{\text{Control abs.} - \text{Test abs.}}{\text{Control abs.}} \times 100$$

Determination of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity
Each extract and fraction (0-20 mg/ml) dissolved in deionised water (2 ml), was mixed with 2 ml of methanolic solution containing DPPH radicals, resulting in a final concentration of 0.1 mM DPPH. The mixture shaken vigorously and left to stand for 30 min in the dark, and the absorbance then measured at 517 nm against a blank[15]. Ascorbic acid used as the positive control. The percentage scavenging calculated as:

$$\% \text{ I scavenging} = \frac{\text{Control abs.-Test abs.}}{\text{Control abs.}} \times 100$$

$OH^{\bullet}$ scavenging assay
OH- scavenging ability measured according to a literature procedure [16] with a few modifications. OH- radicals generated from FeSO4 and H2O2, and detected by their ability to hydroxylate salicylate. The reaction mixture (3 ml) contained 1 ml FeSO4 (1.5 mM), 0.7 ml H2O2 (6 mM), 0.3 ml sodium salicylate (20 mM) and varying concentrations of extract and fractions. After incubation for 1 hour at 37°C, the absorbance of the hydroxylated salicylate complex measured at 562 nm. The percentage scavenging effect calculated as:

$$\% \text{ I scavenging} = \frac{\text{Control abs.-Test abs}}{\text{Control abs.}} \times 100$$

***In-vivo* Antioxidant Activity**
Inhibition of lipid peroxidation in rat liver homogenate Preparation of rat liver homogenate: Albino rats (180-200 g) of either sex kept in mentioned conditions, after the rats got acclimatize to the provided conditions they selected for the study. On the day of experiment, all rats sacrificed under light anesthesia and their liver dissected out. The liver lobes washed properly from tris buffer (pH 7.2) and evenly crushed. The liver was thoroughly homogenate with the help of mortar and pistil with the aid of tris buffer of pH 7.2. After homogenizing rat liver, it allowed to centrifuge at 3000 rpm for 10 minutes. The supernatant containing liver cells used for the study.

RESULTS
Preliminary phytochemical screening The phytochemical investigation in this plant showed that this contains variety of phytochemical especially we found that the ethanolic extract and ethyl acetate and water fractions of leaves of *A. paniculata* contain diterpenoids, phenolics, flavonoids, saponins and tannins. TLC and co-TLC profiles of this plant showed that it contains rutin and quercetin as one of the major flavonoids.

Antiulcer Activity
These screening models for gastric protections are significant to investigate the protective measures of any synthetic or herbal origin drugs and are popular amongst the researchers. The present study has been in order to investigate the protective effect of *A. paniculata* against gastric/peptic ulcer. The stress-induced ulcer has used to investigate the protective effect of drug against ulcer induced by various kind of stress. Gastric lesions induced by HCl/ethanol The present study done with using EA, EAC and PPF of *A. paniculata* leaves. The protection against
the peptic ulcer has observed by calculating the parameter ulcer index and percent inhibition. An acute dose of extract 500 mg/kg of body weight; showed 64 % ulcer inhibition (% I) and the Ulcer index (UI) of it was found to be 20.44±3.49** ($p<0.01$) as compared to control which has the UI 64.21±1.49. The EAC fraction treated animals showed good response but was not significantly good as

compared to any of the drug treated groups the mean UI of EAC was found to be 20.44±3.49** (p<0.01) and the % I at a dose of EAC (50 mg/kg) was 53 %. The UI and % I of ppt. fraction was excellent when compared to the standard drug. The UI of PPF (50 mg/kg) was found to be 4.00±0.54** (p<0.01) and the percent inhibition was came out to be 93 % which was quite close to the standard treated group. Standard Ranitidine had given an acute dose of 100 mg/kg showed 95 % inhibition and the UI of 2.94±2.03. These results illustrate that ppt. fraction was most potent as compared to other two plant drug treated groups (Table 1).

Table 1: Effects of alcoholic extract and their fractions of *A. paniculata* leaves on gasric ulceration by HCl/Ethanol

Group	treatment	Dose (mg/kg)	UI	% I
I	control	-	64.21±1.49	-
II	EA	500	20.44±3.49**	64%
III	EAC	50	29.98±0.53**	53%
IV	PPF	50	4.00±0.54	93%
V	Ranitidine	100	2.94±2.03	95%

Data expressed as mean±S.E.M (n=6), **p<0.01; EA: Ethanolic fraction; EAC: Ethyl acetate fraction; PPF: Precipitates fraction.

Stress- induced acute gastric lesion Stress is one of the causes of the generation of various diseases amongst all; ulcer is one of them. Hypothermic stress induced ulcer model showed kind of same results shown by the HCl/Eth. induced model except for EAP treated fraction. In this study to the ppt. fraction showed significant reduction in the gastric lesions. An acute dose of extract 500 mg/kg of body weight; showed 71 % of ulcer inhibition and the Ulcer index of it was found to be 12.50±3.56** (p<0.01) as compared to control which showed the UI 54.00±3.08. The EA fraction treated animals showed significantly good response, which was descent, compared to the ext. treated group. The mean UI of EA was found to be 12.10±1.20** (p<0.01) the % I (50 mg/kg) was 78 %. The UI and % I of ppt. fraction was very good when compared to the standard drug. The UI of ppt. fraction at an acute dose of 50 mg/kg was found to be 4.54±1.03** (p<0.01) and the percent inhibition came out to be 92 % which was quite close to the standard treated group. Standard Ranitidine when given an acute dose of 100 mg/kg showed 99 % inhibition and the UI of it was very low as compared to all groups 0.50±0.34 (Table 2).

Table 2: Effects of alcoholic extract and their fractions of *A. paniculata* leaves on stress-induced ulceration

Group	treatment	Dose (mg/kg)	UI	% I
I	control	-	54.21±1.49	-
II	EA	500	12.50±3.56**	71%
III	EAC	50	12.10±1.20**	78%
IV	PPF	50	4.54±1.03	92%
V	Ranitidine	100	0.50±0.34	99%

Data expressed as mean±S.E.M (n=6), **p<0.01; EA: Ethanolic fraction; EAC: Ethyl acetate fraction; PPF: Precipitates fraction.

$O_2^{\bullet -}$ scavenger activity assay

The present in vitro antioxidant activity done in order to investigate the O_2 radical scavenging activity of plant and it compared with the standard antioxidant i.e. ascorbic acid. We studied this activity in the function of time. 10 mg/ml of ethanolic extract (100 µl in reaction mixture) scavenging was in the order of 49.9, 47.2 and 42.5 % in 15, 30 and 45 min. respectively, which was more, then EAC. The percentage reduction of O_2 radicals by EAC (10 mg/ml) was 33.0 % in 15 min; 32.7 % in 30 min and was 32.0 % in 45 min. The PPF (10 mg ml) showed excellent reduction in the generated radical which found to be 78.0, 68.8 and 67.0 % in 15, 30 and 45minutes, which was quite good when this compared to the positive control that was ascorbic acid (10

mg/ml). The percentage reduction of ascorbic acid came out to be 96.0 % in 15 min. 92.2 % in 30 min. and was 90.75 % in 45 min.

DPPH radical-scavenging activity

Two-fraction viz. EAC and PPF showed good results when compared to the positive control. In the conc. Range of 2-20 mg/ml both showed percentage reduction of 41.9-71.0% and 58.0-73.8% respectively. The extract of the plant in the same concentration range showed good percentage inhibition i.e. 47.9 to 71.0% in 2-20 mg/ml Ascorbic acid showed an excellent scavenging (83-94.4 %). It observed that ppt. fraction also had strong scavenging activity.

OH-scavenging activity

The percentage scavenging found to be increasing with the increase of concentration. Amongst all, ppt. fraction showed very good scavenging activity as shown in the table. The percent inhibition of ascorbic acid (positive control) was greatest in he tested concentration range 2-20 mg/ml as it inhibited 81.7-89.0% of OH radicals. In same conc. range, ppt. fraction showed 68-77.7% inhibition compared to extract and EA fraction, which showed 40.0-67.3 and 39.3-53.0% inhibition (Table 3).

Table 3: Effects of *A. paniculata* leaves alcoholic extract and their fractions of on percentage inhibition by OH-scavenging activity

Group	Scavenging agent	Extract (mg/ml)					
		2	6	8	10	12	20
I	control	-	-	-	-	-	-
II	EA	40.0%	40.5%	43.3%	43.7%	53.9%	67.3%
III	EAC	39.3%	40.3%	40.7%	42.1%	44.4%	53.0%
IV	PPF	68.0%	69.1%	69.8%	71.7%	71.0%	77.7%
V	standard	81.7%	83.0%	83.2%	83.7%	83.7%	89.0%

Data expressed as mean±S.E.M (n=2), EA: Ethanolic fraction; EAC: Ethyal acetate fraction; PPF: Precipitates fraction.

DISCUSSION

It is quite evident that this plant may prove very useful medicinally. The present work has opened the gates to investigate this plant phytochemically and pharmacologically. We found that the ethanolic extract and ethyl acetate and water fractions of leaves of *A. paniculata* contain diterpenoids, phenolics, flavonoids, saponins and tannins. It contains rutin and quercetin as one of the major flavonoids. Earlier investigations showed that this plant contains variety of flavonoid compounds. Although in most of the cases the etiology of ulcers is unknown, it generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defense mechanisms. To regain the balance different therapeutic agents including plant extracts have used to inhibit gastric acid secretion or to boost the mucosal defense mechanism by increasing the mucus production, stabilizing the surface epithelial cells, or interfering with PGs synthesis. Ethanol is widely used to induce experimental gastric ulcer in animals. For the purpose of lesion formation, administration of ethanol utilized since it easily and rapidly penetrates into the gastric mucosa. By increasing mucosal permeability and the release of vasoactive products, ethanol causes vascular damage and gastric cell necrosis, which, in turn, leads to ulcer formation. Also due to good antioxidant activity, the extract may inhibit tissue-derived mediators, which cause cellular necrosis, thereby exhibiting antiulcer activity.

The extract exhibited a significant protective effect on gastric mucosa against stress-induced ulceration. During stress vagal over activity leads to histamine release, which increases the acid secretion. Since mast cell membrane stabilizers possess antiulcer activity by reducing the production of oxygen free radicals, it is likely that the plant extract and fractions may act as a mass cell membrane stabilizer. This effect may be because of the polyphenolic such as tannins, flavonoid content of this plant several studies have revealed that polyphenolic compounds have potent antiulcer activity and they may work as an antioxidants and may be because of these might be showing the antiulcer activity. Mucosal damage can easily produced by the generation of active oxygen and free radicals. Ethanol increases superoxide anion, hydroxyl radical production and lipid peroxidation in the gastric mucosa. The tissue damage to the gastrointestinal mucosa,

induced by acute and chronic ethanol toxicity, may be associated with the generation of toxic species that disturbs the balance of oxidant/ antioxidant cellular processes. Acute ethanol treatment induces oxidative stress, DNA damage, increased xanthine oxidase activity and malondialdehyde levels, and decreased total GSH content in gastric mucosal cells. The antiulcer pharmacological effects of several plants related to their flavonoid content. On the other hand, tannins as flavonoids are polyphenols, and their antiulcer activity may have a similar mechanism of action. Among the principal properties that may account for the potential benefits of flavonoids, is their antioxidant activity, which also be presented by tannins, including *A. paniculata* .

A. paniculata through all the antioxidant activities screened reveals that it got antioxidant potential or free radical scavenging potential, so there one more underlying mechanism for the antioxidant and antiulcer activities may be through later. Based on available results we can assume that this plant may be giving both antioxidant and antiulcer activities because of one or several mechanisms. The antioxidant effect of this drug in conclusion may be because of the free radical scavenging effect as it was mentioned earlier that flavonoids causes reduction in the generated radicals as they contain catechol nucleus. The gastro protective effect of this plant is may be because of quercetin and several other flavonoids increases the sodium and potassium ions flow in lumen. Due to the tannin content of this plant, it may say that due to the astringent effect and antioxidant activity of latter this effect may be possible. Tannins may be competing with H_2 receptor antagonizing and reversibly competing with histamine like the H_2 receptor antagonists like Ranitidine, which reduces gastric secretion and blocks the acid secretion stimulated via histamine secretion and gastric hormone. The stress induced ulcer protective effect of this drug may be because during stress, vagal over activity causes histamine release and increases acid secretion via histamine. Mast cell stability provided by flavonoids may be the other reason as mast cells generate the O_2 radicals.

From the above investigations it revealed that extract and different fractions of *A. paniculata* was bring into being to scavenge DPPH, OH^-, O_2^- and MDH with high reducing ability. This activity was owing to flavonoids and tannins present therein as revealed by the quenching of the DPPH radical. The antioxidant activity can endorsed due to the existence of different fractions, which has confirmed by phytochemical tests.

In summary, our data provide evidence that extract and different fractions of *A. paniculata* act as both antioxidant and antiulcer agents. Although further investigation will be required to discover the precise mechanism by which extract and different fractions act, our results are a key to demonstrating the scientific foundation of the traditional and folkloric medical uses of the *A. paniculata* plant in terms of its antioxidant and antiulcer activity.

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