Antiulcerogenic activity of hydromethanolic extract of Andrographis paniculata in Indomethacin and Indomethacin plus pylorus ligation induced gastric ulcer in rats.

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Abstract
Ethnopharmacological relevance: Andrographis paniculata (Acanthaceae) is given from ancient times in Indian traditional medicine like Ayurveda and Unani for the treatment of gastrointestinal tract disorders, bronchial diseases, fevers, inflammatory diseases, liver disorders, parasitic diseases and snake poisoning.

Aim of the study: Investigation of hydromethanolic extract of Andrographis paniculata in the treatment of ulcer by Indomethacin and Indomethacin plus pylorus ligation induced gastric ulcer in rats.

Materials and methods: The antiulcer activity of hydromethanolic extract of Andrographis paniculata (APE) was investigated using 36 rats. The first group was subjected as control, the second group was subjected to pylorus ligation on 6th day under ether anesthesia, and the third group was subjected to Indomethacin (20 mg/kg) for 5 days + pylorus ligation on 6th day under ether anesthesia. The fourth and fifth groups were administered with the 50% hydromethanolic extract of Andrographis paniculata (200 and 400 mg/kg/day, respectively). The sixth group served as reference antiulcer drug omeprazole (10 mg/kg/day). All animals were deprived of food (but not water) for 24 hours prior to being subjected to ulcerogenic challenge. At the end of study, the stomach tissue was cut, washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution for histopathological examination. Ulcer Index, pH, titrable acidity, gastric mucus, antioxidant activity, and gastric pepsin activity was evaluated by using tissue and gastric juice.

Results: APE showed dose-dependent ulcer protective effect in indomethacin plus pylorus ligation induced gastric ulcer. The % protection was found in group IV (19.32% & 30.75%), group V (62.17% & 67.47%) and group VI (70.38% & 74.57%) when compared to PL group II and IND+PL group III, respectively. The APE showed highly significant enhancement of gastric wall mucus at the dose of 400 mg/kg. The TBARS level was significantly increased in group II rats and IND+PL group III rats respectively, while the level of GSH was significantly decreased as compared to control group. The pH was significantly increased with subsequently decrease in acidity with the treatment of APE. APE showed a marginal increase in pepsin activity compared to control group I rats. These observations were in good corroboration with the histopathological studies results.

Conclusions: The results of our study revealed that the extracts of Andrographis paniculata possess significant dose dependent gastroprotective and antisecretory effects by strengthening the gastric mucosa, decreasing the acidity of gastric juice and pepsin activity as well as restore the imbalance antioxidant activity. Further studies on other factors like H. pylori, PGs and cAMP, which play important role in ulcerogenesis may provide more insights on the antiulcerogenic activity of Andrographis paniculata.

Keywords: Andrographis paniculata; Indomethacin; Pylorus ligation; Ulcer index; Gastric output; Omeprazole.
INTRODUCTION
The Gastric ulcer is a major problem affecting the 4-10% of population worldwide with its pervasiveness is quite high in India. The peptic ulcer originates with unknown reason. The peptic ulcer is induced by stress, smoking, nutritional deficiencies, ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs), hereditary predisposition and infection by Helicobacter pylori [1]. Some studies have shown that duodenal ulcer is more common in the Southern part of India compared to the North [2]. The imbalance between aggressive and protective factors in the stomach may provoke ulcers [3-5]. The current treatment of gastric ulcers is based mainly on antacids, anticholinergics, proton pump inhibitors and H2-receptor antagonists. The major drawbacks of these treatments are intolerance, excessive development of breasts in males, erectile dysfunction, cardiac arrhythmia and haematological disorders. Thus, there is an emergent need to develop the alternative therapies for the treatment of gastric ulcers. One of the most promising source of new treatments for this complaint is plant extracts [6-7].

Andrographis paniculata (Burm. f. Nees Syn: Kalmegh, Acanthaceae), is abundantly found in south-eastern Asia: India, Sri Lanka, Pakistan and Indonesia [8]. Phytotherapeutic studies have revealed that whole herb extract is an effective modality for therapeutic and preventive purposes due to its complex composition and interactions, which may modulate signal transduction and metabolic pathways. The aerial part is used to extract the active phytochemicals. Andrographine, Panicoline, Diterpene dimers Flavonoids were isolated from the roots. The most prominent and active constituents were andrographolides and andrographis [9]. The most biological activities of this plant were attributed to the diterpenoids, flavonoids and quercetin [10]. Andrographolide has also been shown to protect against hypoxia-reperfusion injury in neonatal rat cardiomyocytes. A. paniculata is documented in the literature for a broad category of pharmacological activities such as anti-inflammatory [11], hypoglycemic[12], antidiarrhoeal[13], antiviral [14], antimalarial [15], antineoplastic [16], antihuman immunodeficiency virus (HIV) [17], immune stimulatory [18], and anti snakebite activity[19], toxicity to male reproductive system and anti-cancer enhance the function of cardiovascular (for prevention of atherosclerosis and heart disease [20].

In view of the above, the present study was performed to investigate the antiulcer activity of the hydromethanolic extract of A. paniculata. The effects of this plant were also confirmed by studying its in vivo antioxidant effects; antioxidants, such as malondialdehyde and glutathione, seem to have protective roles against gastric ulcers.

MATERIALS AND METHODS
Drugs and Chemicals: The standard drugs viz. Omeprazole (Sd fine-Chem. limited), acetic acid (Rankem ltd.), methanol (Fisher Scientific), indomethacin (Jagsonpal), 5,5-dithiobis-2-nitro benzoic acid (Sigma-Aldrich), Alcian Blue and Albumin were from Sd fine-Chem. Limited were used for evaluation of the study. The AR grade chemicals and buffers were used and the buffer solutions were prepared immediately before use.

Preparation of plant extract: The commercially available entire aerial plants of A. paniculata was obtained from local market of Lucknow, and authenticated by National Botanical Research Institute (NBRI) Lucknow and a voucher specimen (NBRI-SOP-202) was deposited for future references. The powdered aerial parts of A. paniculata first defatted with Petroleum ether (60-80 °C). The defatted marc was dried under shade to get a dry mass, which was then extracted with Methanol and water (50% hydromethanolic) by using cold maceration extraction continued for 2 days at room temperature with intermittent shaking solvent was filtered, concentrated on rotavapour (Buchi, USA). The final extract obtained was weighed calculated for percentage yield (18.97% w/w) and stored in a cool place.

Animals: Wistar rats (200 – 250 g) were procured from the Animal House Facility, Faculty of Pharmacy, Integral University, Lucknow. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (temperature and relative humidity was maintained at 22 ± 2 °C and 50 ± 15 % respectively and 12 h light/dark cycle) and had a free access to standard pellet diet (Dyal Industries, Barabanki, Lucknow, India) and drinking water ad libitum. The animals were randomized in to experimental and control groups. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC), Regd. No.IU/Pharm/M.pharm/CPCSEA/12/14.

Antiulcer activity: The antiulcer activity of Andrographis paniculata was investigated using six groups. The first group was subjected as negative control received 1% CMC (1 ml/kg, p.o.) for 5 days, the second positive control group was subjected with the 1% CMC (1 ml/kg, p.o.) for 5 days + pylorus ligation [21] on 6th day beneath anesthesia, and the third group was subjected to Indomethacin (20 mg/kg) for 5 days + pylorus ligation on 6th day beneath anesthesia. The fourth and fifth groups were administered with the 50% hydromethanolic extract of A. paniculata (200 and 400 mg/kg/day, respectively). The sixth group was treated with omeprazole (10 mg/kg/day). All animals
were deprived of food (but not water) for 24 hours prior to being subjected to ulcerogenic compounds.

**Ulcer Index:** Ulcer Index was resolute according to the score method of [21]. The lesions of each stomach were used for the calculation of ulcer index (UI), and the protection percentage was calculated from the following formula: \[
\frac{[\text{UI control} - \text{UI treated}]}{\text{UI control}} \times 100
\]

**Determination of gastric mucus:** The gastric tissues were instantly transferred to 10 ml of 0.1% w/v alcian blue solution. Mucus was twice rinsed with 250mM sucrose solution at 15 and 45 min. Mucus and dyes complexes was extracted with 10 ml of 500mM magnesium chloride solution and was occasionally shaken for 1 min for 2 h at a interval of 30 min. The resulting solution was mixed with diethyl ether in an equal volume and centrifuged for 10 minutes at 3000 r.p.m. Absorbance was taken at 580nm. The quantity of mucus was calculated by standard curves of alcian blue (Ranging from 20 µgm/10 ml), and the results were articulated in µgm of Alcian Blue/g tissue [22].

**Estimation of Thiobarbituric Acid Reactive Substances (TBARS):** The gastric tissue was rinsed, scraped, weighed and homogenized in 0.15 M KCl solution (1:10, w/v). 1 ml of suspension is added with 0.5 ml of 30% TCA and by 0.5 ml of 0.8% TBA reagent. The above solution was transferred in a test tube and covered with aluminium foil and kept on a water bath for 30 min. at 80°C and cooled in ice-cold water for 30 minutes thereafter centrifuged for 15 min. at 3000 rpm. The absorbance of the organic layer was measured at 540 nm [23].

**Estimation of Tissue Glutathione:** 500 mg of tissue was homogenized in 10 ml of 200mM potassium phosphate buffer (pH6.5) 50% trichloro acetic acid is added to the aliquot homogenate and vortex it for 10 min. and then centrifuged at 6000rpm. The supernatant was taken out and 0.4 M Tris buffer nad 0.01 M DTNB was added to it. The absorbance of the supernatant was observed at 415 nm [24].

**Determination of free acidity and total acidity:** 1 ml of gastric juice is diluted with 10ml of distilled water, 2 drops of Topfer’s reagent was added and titrated with 0.01 N sodium hydroxide until end point reached. The used volume of NaOH corresponds to free acidity. Then 2 - 3 drops of phenolphthalein reagent was added and titration was continued until a red tinge reappears [25]. The total amount of sodium hydroxide corresponds to total acidity. Acidity was calculated by using the following formula:

\[
\text{Acidity} = \text{Volume of NaOH} \times \text{Normality of NaOH} \times 100/0.1 \text{mEq.L/100 gm}
\]

**Estimation of Gastric pH:** The gastric content earlier collected was used for the determination of pH by using a digital pH meter [26].

**Estimation of Gastric Pepsin activity:** According to that 0.2 ml of centrifuged gastric juice plus 3 ml of albumin 3% for each rat test and blank. After that 10 ml of 6% trichloroacetic acid was added to blank to stop enzyme activity. Both blank and test tubes was incubated in water bath at temperature 37°C for 30 minutes. Then 10 ml of trichloroacetic acid was added to test tubes, srhlken well and filtered. Proteolytic activity was determined spectrometrically by optical density at 280 wave length. Pepsin content was determined by extrapolation with standard curve [27].

**Histopathology:** At the end of study, the stomach tissue was cut, rinsed with cold saline. The tissue was fixed in 10% buffered formalin solution. The slides were observed under light microscope and photomicrograph was taken and shown in figure 7.

**Statistical analysis:** Results were expressed as mean ± SEM. The Data were analyzed by student-’t’ test with multiple comparisons with the groups. The P value 0.05 was considered to be statistically significance.

**RESULTS**

**Extractive value phytochemical test:** The hydromethanolic extract was prepared by cold maceration. The yield for the same was found to be 18.97% w/w.

**Ulcer index:** The anti-ulcer potential of the new drugs can be investigated by using the ulcer index which is a measure of gastric mucosal lesion. The drug or ulcerogen induced gastric damage was marked by the mucosal lesions and spots of different sizes.

The group II animals with pylorus ligation rats showed significant increase in the ulcer index (P < 0.001) when compared to group I rats. IND+PL group III rats showed significant increase in the ulcer index (P < 0.001 & P <0.05) when compared to control group I and group II rats, respectively. The group IV and V showed significant decrease in the ulcer index (P < 0.05 and P < 0.001) as compared to the group II animals at 200 and 400 mg/kg APE extract. In contrast, the APE treated group IV, V and VI shows significant decrease in ulcer index (P<0.001) when compared to group III. Figure 1 shows the effects of APE. The % protection was found in group IV (19.32% & 30.75%), group V (62.17% & 67.47% %) and group VI (70.38% & 74.57%) when compared to PL group II and IND+PL group III, respectively.

**Gastric wall mucus (barrier mucus determination):** The figure 2 represents the effects of APE on gastric wall. The group II and III showed significant decrease in the gastric mucus with the P<0.001 as compared to control group I. The group III rats showed increased gastric mucus but not significant when compared to control group II rats. The group IV rats at the dose of 200 mg/kg showed significantly high gastric mucus (P < 0.01) as compared
to group II and group III animals respectively while APE treated groups at the dose of 400 mg/kg showed significantly increased gastric mucus (P < 0.001) compared to group II animals. The APE treated group V and VI at 400 mg/kg significantly increased gastric mucus (P<0.001) as compared to group III animals.

**Estimation of Thiobarbituric Acid Reactive Substances (TBARS) and Tissue Glutathione Level (GSH):** The figure 3 represents the effects of APE on TBARS and GSH. The group II and III showed significant increase in TBARS level (P < 0.001) and decrease in GSH levels (P < 0.001) as compared to control group I. In group III rats showed significant increase in the TBARS levels and slight decrease in GSH levels when compared to group II.

The group IV and V showed significant decrease in the TBARS levels (P < 0.05) when as compared to group II at the 200 and 400 mg/kg dose respectively. The group IV and V animals treated with 200 and 400 mg/kg dose significantly decreases the TBARS levels (P < 0.05, P < 0.01) as compared to group III animals. The standard treated group VI significantly decreased TBARS level (P < 0.001) when compared to group II and III animals.

The significant increase in the GSH (P < 0.05 and P < 0.01) in the group IV and V animals was observed when treated with 200 and 400 mg/kg dose of APE as compared to group II. The GSH was found significantly increased (P < 0.01) with the dosing of the animals at 200 and 400 mg/kg of APE when compared to group III animals. The group VI treated with standard drug showed significant increase in GSH (P < 0.01) when compared to group II and III animals.

**Determination of pH and Titratable acidity:** The significant increase in pH and decrease in acidity was observed in the ulcer induced rats pretreated with APE as represented in the figure 4. The group II and III group treated rats showed decreased pH (P < 0.001) as compared to control group I rats while in group IV there is a significant rise in the pH (P < 0.01) was observed when compared to group II and III animals respectively at the dose of 200 mg/kg. In group V there is a significantly increased pH (P < 0.001) when compared to group II and group III animals respectively. The standard treated group VI shows significant increased pH (P < 0.001) when compared to group II and group III animals.

There is an increase (P < 0.01, P < 0.001) in the free acidity in group II and IND+PL treated rats as compared to control group I rats. The group II does not show a significant rise in the acidity when compared to III and group III when treated with APE group IV animals. The APE treated groups IV rats at 200 mg/kg significantly decreased free acidity (P < 0.05) when compared to group III animals. In contrast, the APE treated group V at 400 mg/kg significantly decreased free acidity (P < 0.01) when compared to group II animals group III animals. The standard treated group VI shows significant decreased free acidity (P < 0.01 and P < 0.001) when compared to group II and group III animals, respectively.

The group II and III rats showed increased total acidity (P < 0.01) compared to control group I rats. The APE treated group V at 400 mg/kg rats showed decrease in total acidity (P < 0.05) compared to control group II rats. The APE treated group IV and V at 200 & 400 mg/kg significantly decrease in total acidity (P < 0.05 and P < 0.01) when compared to group III animals. In contrast, the standard treated group VI shows significant decreased total acidity (P < 0.01) when compared to group II and group III animals, respectively.

**Gastric pepsin activity:** The group II and III rats showed remarkable increase in pepsin activity (P < 0.001) as compared to control group I rats fig. 5. Pylorus ligation group II rats showed marked increase in pepsin activity (P < 0.01) compared to control group III rats. In contrast, the APE treated groups IV and V rats at 200 and 400 mg/kg as well as standard treated group VI significantly decreased pepsin activity (P < 0.001) when compared to group II and group III animals, respectively.

Pretreatment with the drug was found to reduce pepsin concentration to near normal level and the effect was comparable to that of standard drug as omeprazole.

![Figure 1](image1.png) Effect of 50% hydromethanolic extract of *Andrographis paniculata* on ulcer index in control and experimental groups of animals.

![Figure 2](image2.png) Effect of 50% hydromethanolic extract of *Andrographis paniculata* on mucus barrier in control and experimental groups of animals.

Figure 3 Effect of 50% hydromethanolic extract of *Andrographis paniculata* on TBARS and GSH in control and experimental groups of animals.

PL+ IND = Pylorus ligation plus Indomethacin.

Values are expressed as Mean ± SEM of six rats in each group.

P value: $<0.001$ when compare to respective control group I.

Ns = not significant when compare to respective group II.

P value: $<0.05$, **$<0.01$** and ***$<0.001$** when compare to respective group II.

P value: $<0.05$, **$<0.01$** and ***$<0.001$** when compare to respective group III.

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Figure 4 Effect of 50% hydromethanolic extract of *Andrographis paniculata* on pH, free acidity and total acidity in control and experimental groups of animals.

PL+ IND = Pylorus ligation plus Indomethacin.

Values are expressed as Mean ± SEM of six rats in each group.

P value: $<0.01$ and $<0.001$ when compare to respective control group I.

P value: $<0.05$, **$<0.01$** and ***$<0.001$** when compare to respective group II.

P value: $<0.05$, **$<0.01$** and ***$<0.001$** when compare to respective group III.

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Figure 5 Effect of 50% hydromethanolic extract of *Andrographis paniculata* on pepsin activity in control and experimental groups of animals.

PL+ IND = Pylorus ligation plus Indomethacin.

Values are expressed as Mean ± SEM of six rats in each group.

P value: **$<0.01$** and ***$<0.001$** when compared to respective control group I.

P value: **$<0.01$** and ***$<0.001$** when compared to respective control group II.

P value: ***$<0.001$** when compare to respective group III.

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DISCUSSION

The current therapy of peptic ulcer is expensive, limiting their use and have side effects too. Therefore the search for more effective, nontoxic and inexpensive antiulcer drug is a challenging task. The medicinal plants are good source of new drugs. *Andrographis paniculata* is a medicinal plant widely used by the population as a remedy for gastric problems. Literature
The study at APE in a dose
The gastric mucosal integrity is depending on the balance between HCL pepsin and the protective factors as mucus and HCO³⁻ secretion, prostaglandins, mucosal blood flow, nitric oxide [28]. Therefore the main guidelines for the treatment are aimed to block the acid secretion, but also on the increased production of factors responsible for protecting the gastric mucosa, thus avoiding damage to the epithelium [29]. The present study shows the protective effect of Andrographis paniculata due to decrease in ulcer formation.

Ligation of pylorus causes beginning due to existence of acid and pepsin in abdomen [21] which leads to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier [30].

The indomethacin induced model which was well documented in the literature was used in this study [31]. Prostaglandins shows protective effect on stomach mucosa and causes increase in bicarbonate secretion, maintain mucosal blood flow and repair thus increase in mucosal lesions is caused by suppressing prostaglandin synthesis by NSAIDs [32]. Hence, Indomethacin plus pylorus ligation model was used in our study to induce severe ulceration in rats.

The study shows the effects of APE against indomethacin plus pylorus ligation induced gastric ulcers significantly decreased in gastric content, pH and total acidity. APE at a dose of 200 and 400 mg/kg shows decreased in ulcer index and score. Due to presence of phenolic compound in APE the pretreated experimental rats’ shows considerable decrease in strength of gastric mucosal damages (p < 0.001) in a gradient manner [33-34]. Due to their free-radical scavenging and antioxidant properties of phenolic compounds they cause augmentation of mucus production and anti inflammatory action [35].

Gastric lesion is a result of an imbalance between defensive and aggressive factors which leads to the formation of ulcer. Thus, the mucus on the tissue was quantified, and result shows that APE in a dose dependent manner causes enhancement of mucus production [36-37]. Thus, APE has possible function in enhancing mucosal resistance to gastric acid and shows a protective effect on experimental ulcer [38].

Increased formation of reactive oxygen metabolite shows role in the pathogenesis of various inflammatory condition including abdominal disease and peptic ulcer. The result of the present investigation showed that the elevation in the level of TBARS in indomethacin plus pylorus ligation induced rats was significantly minimized by APE pretreated groups of rat. This might be due to the lipid peroxidation preventing role of the APE during ulcerogenesis. APE has been shown to enhance antioxidant potential in experimental animals. The APE contain affluent quantity of flavonides viz quercetin, Biochin A and Formononetin and these flavonides claimed for anti lipid peroxidative effect observed in this study [39].

A major element of the intracellular protective mechanism against a many noxious stimuli, including oxidative stress is glutathione. The excessive free radical generation (oxygen radicals in the extracellular space and depletion of glutathione) aided with the inhibition of glutathione peroxidase are responsible for oxidative tissue damage of the gastric mucosa [40-41].

In our study, significant decreased in glutathione concentrations shown in control group II and group III, whereas the rats pretreated with hydromethanolic extract of Andrographis paniculata showed a significant increase in the glutathione level, suggesting that these extracts prevent the depletion of nonprotein sulfhydryl groups caused by indomethacin and pylorus ligation treatment [42].

The gastric ulcers in the pylorus is mainly due to increased gastric HCL secretion leading to destruction of mucosal barrier and autodigestion of gastric mucosal layer. The suggested treatment for this condition may include Proton pump inhibitors (PPIs) acting through the inhibition of the H⁺ K⁺ ATPase enzyme activity [43]. Investigation of hydromethanolic extract of Andrographis paniculata showed significant inhibitory effect on the total acidity and free acidity. Our findings is also supported by [44] the ulcer preventing effect APE is due to its regulating effect on H⁺ K⁺ ATPase activity and/or mucin preserving effects.

In ulcer induced animals there is significant increased in level of acid and pepsin [45] and indomethacin also increases pepsinogen secretion [46]. In this study we have found that, there are significant increase in pepsin activity after pylorus ligation and indomethacin administration. Pretreatment with APE shows reduction in pepsin level when compared with omeprazole.

It was observed that the APE has anti inflammatory property, as experimented by decrease in total and free acidity. Further the APE offers cytoprotection by enhancing mucus wall thickness and antioxidant activity. The gastric prophylactic and curative effects of APE may be predominantly due to its activity on defensive mucosal factors. The inherent antioxidant activity of APE may be one of the important factors contributing towards its activity.

CONCLUSION: The present study strongly demonstrated that 50% hydro-methanolic extract of Andrographis paniculata possess significant dose dependent gastroprotective activity in pylorus ligation
induced gastric ulcer with or without indomethacin in rats, by strengthening the gastric mucosa, decreasing the acidity of gastric juice and pepsin activity as well as restore the imbalance antioxidant activity. These findings could justify, at least partially, the ethnomedicinal use of this plant in the management of gastric disorders. The findings of this experimental study could lead to further isolation, and pharmacological activity of new therapeutic compounds effective against ulcer.

CONCLUSION: The present study strongly demonstrated that 50% hydro-methanolic extract of *Andrographis paniculata* possess significant dose dependent gastroprotective activity in pylorus ligation induced gastric ulcer with or without indomethacin in rats, by strengthening the gastric mucosa, decreasing the acidity of gastric juice and pepsin activity as well as restore the imbalance antioxidant activity. These findings could justify, at least partially, the ethnomedicinal use of this plant in the management of gastric disorders. The findings of this experimental study could lead to further isolation, and pharmacological activity of new therapeutic compounds effective against ulcer.

REFERENCES:


