ORIGINAL ARTICLE

Pharmacological *in-vitro* investigation for digestive property and *in-vivo* anti-ulcer activity of Pep-Up Syrup

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Objective: To investigate *in-vitro* digestive property and *in-vivo* anti-ulcer activity of Pep-Up Syrup in aspirin-induced acute gastric ulcer. **Materials and Methods:** The *in-vitro* investigation for digestive property was performed by means of evaluating amylolytic, lipolytic and proteolytic activity in comparison with blank. For *in-vivo* evaluation of Pep-Up Syrup, aspirin-induced acute gastric ulcer model in rats was used. Selected animals were divided in to three groups where each group was consisting of six animals. Group-I, II and III was considered as normal control, disease control and Pep-Up Syrup treated, respectively. Pep-Up Syrup (3 mL/kg/day) treatment was provided for 7 days orally. Ulcer index, gastric wall mucus content, lipid peroxidation level in stomach tissue and tissue antioxidant parameters like superoxide dismutase (SOD), reduced glutathione and catalase enzyme activity were carried out. Histopathology of stomach tissue was also performed. **Statistical Analysis:** Statistical calculations were done by analysis of variance (ANOVA) followed by *post-hoc* Bonferroni's test and results were expressed as Mean ± SEM. **Results and Discussion:** Pep-Up Syrup showed noticeable amylolytic, lipolytic and proteolytic activity. Pre-treatment of Pep-Up Syrup showed significant protection against ulceration and aspirin-induced loss of gastric wall mucus content. Pre-treatment of Pep-Up Syrup also showed significant increase in tissue antioxidant parameters. Histopathology study revealed that Pep-Up Syrup provides significant cyto-protection against aspirin-induced mucosal damage. **Conclusions:** On the basis of study data it can be concluded that Pep-Up Syrup possesses property of digesting starch, lipids and proteins. Data also revealed that Pep-Up Syrup has antiulcer activity against aspirin-induced acute gastric ulcer.

Key words: Antioxidant, *in-vitro* digestive property, *in-vivo* anti-ulcer activity, Pep-Up Syrup

INTRODUCTION

Gastrointestinal (GI) problems are the most common and widespread health complaints among the general populace. Indigestion (dyspepsia) is a vague feeling of discomfort in the upper belly or abdomen. This may include a feeling of heat, burning or pain in the area between the navel and the lower part of the breastbone. Indigestion may be triggered by eating spicy, fatty or greasy foods, eating too fast, overeating, drinking too much alcohol, emotional stress, etc., Other causes of indigestion are gallstones, gastritis, stomach or intestinal ulcers, use of drugs such as antibiotics, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs).[1] According to Ayurveda, the word *Ajirna* means bad digestion. It is defined as a pathological condition in which food is not digested easily and is the root cause for many internal diseases (metabolic). In conventional medicine, the term dyspepsiasis, derived from the Greek words *dys* (bad) and *pepsis* (digestion), refers to symptoms thought to originate in the upper GI tract.[2]

Ulcer is one of the most common GI diseases. It is a lesion in the mucosa of the stomach (gastric ulcer) or duodenum (duodenal ulcer). Ulceration occurs majorly due to imbalance between offensive acid-pepsin secretions versus impaired mucosal resistance, caused most commonly by *Helicobacter pylori* (*H. pylori*) infection and NSAIDs use. Morbidity and mortality from this condition are mainly related to abdominal discomfort and hemorrhage or perforation in stomach.[3] In Ayurveda, peptic ulcers or gastro-duodenal ulcers generally refer to *Parinamasula*.[4]

Dyspepsia is common disease of GI tract. Surveys in Western societies have recorded a prevalence of it between 23 and 41%. The prevalence of dyspepsia ranges from 26% in the U.S. and 41% in the U.K. Although only 20-25% of persons with dyspepsia seek medical care the problem is responsible for 2-5% of visits to primary care physicians.[5] The prevalence of *H. pylori*

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varies by geographic location, socioeconomic conditions, ethnicity, and age. In developing countries, *H. pylori* prevalence exceeds 80% in adults and correlates with lower socioeconomic conditions. In industrialised countries, the prevalence of *H. pylori* in adults is between 20% and 50%. The prevalence of *H. pylori* in the United States is 30-40%.[6]

In India, 49.40% population is suffering from digestive problems among them 21.10% is aging between 12 and 20 years and 76.20% is aging above 70 years.[7]

For the prevention and treatment of peptic ulceration, research advances during last decade have offered new insights. Although drug treatment for peptic ulceration has improved, the need of better therapy is still prevailing. In this situation, medicinal plants may provide an alternative of new drugs. Indian system of medicine is a rich collection of knowledge on traditional medicine which narrates many plants having digestive and anti-ulcer properties.[8]

Pep-Up Syrup is such an Ayurvedic formulation which contains extract of *Withania somnifera* (Ashwagandha) Root,[9] *Plumbago zeylanica* (Chitrak) Root,[10,11] *Apium graveolens* (Amjoda) Fruit,[12,13] *Eclipta alba* (Bhringraj) Whole plant,[14] *Centella asiatica* (Madukparni) Whole plant,[15,16] *Zingiber officinale* (Shunthi) Rhizome,[17,18] *Piper longum* (Pippali) Fruit,[19,20] *Punica granatum* (Dadim) Fruit bark,[21] *Alpinia galanga* (Kulinjan) Rhizome,[22] *Elettaria cardamomum* (Elaichi) Fruit,[23] *Mesua ferrea* (Nagkesar) Stamens[24] and *Cinnamomum zeylanicum* (Twak) Bark.[25] It is a proprietary Ayurvedic medicine manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. Majority of ingredients of Pep-Up Syrup are well reported in Ayurvedic texts and scientific research publications for digestive property and anti-ulcer activity. However, no such evidence was found which proves efficacy of such combination.

In the present study, an attempt was made to investigate *in-vitro* digestive property and *in- vivo* anti-ulcer activity of Pep-Up Syrup in aspirin-induced acute gastric ulcer.

MATERIALS AND METHODS

Experimental Animals

Wistar albino rats of 200-250 g were used and acclimatised to the experimental room having ambient temperature (23 ± 2°C), controlled humidity (55 ± 5%) conditions, and 12 h light and dark cycle. Animals were caged in polypropylene cages with maximum of three animals per cage. The rats were fed with standard food pellets and water *ad libitum*. Study was conducted after obtaining approval by Institutional Animal Ethical Committee (IAEC) (Babaria Institute of Pharmacy, M.Pharm Sem-IV/12-13/15) as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Administration of Test Drug and Dosage

The test drug (i.e., Pep-Up Syrup) was received from Vasu Healthcare Pvt. Ltd., Baroda, Gujarat, India. It was administered orally as it is. Dose of the test drug was fixed by extrapolating the human dose to laboratory animals, based on body surface area ration as per the table of Paget and Barnes.[26]

Determination of *In-Vitro* Digestive Property

Ten milliliters of Pep-Up Syrup was mixed with 20% aqueous glycerol and phosphate buffer (pH 7.8) in ratio 1:4. Mixture was then filtered using simple filter paper and filtrate was used as enzyme source for determination of different enzymatic activities. The samples of standard enzymes were prepared similarly to the test sample.

Amylolytic Activity

One millilitre of starch solution (soluble starch 1% in phosphate buffer) was pipette out in a test tube followed by 1 mL of properly diluted enzyme. It was incubated at 27°C for 15 min. The reaction was stopped by addition of 2 mL of di-nitro salicylic acid reagent and heated in boiling water bath for 5 min. While the tubes were warm, 1 mL of 40% potassium sodium tartrate solution was added then they were cooled down under running tap water. Volume was made up to 10 mL with water and the absorbance was measured at 520 nm. A unit of amylase is expressed as U/mL of maltose produced during 5 min incubation with 1% starch solution.[27]

Lipolytic Activity

Preparation of Substrate Solution

Two milliliters of castor oil was, neutralised to pH 7 and stirred well with 25 mL of water in the presence of 100 mg of bile salts (sodium taurocholate) till an emulsion was formed.

Procedure

Two millilitres substrate was taken in test tube followed by 5 mL phosphate buffer at pH 7. The contents were stirred slowly in magnetic stirrer and the temperature was maintained at 35°C. The electrode of the pH meter was dipped in the reaction mixture and the pH was adjusted to 7. The test enzyme solution (0.5 mL) was added immediately and no pH alteration was observed. The timer was set at zero at this moment. Then pH was brought up to 0.2 units by addition of N/10 NaOH. The pH was observed coming to initial value due to enzymatic reaction. Again N/10 NaOH was added to increase the pH up to 0.2 units. This cycle was continued till reverse of pH to normal was stopped. The time

was noted for this period. The volume of alkali consumed at each time was noted. A unit of activity of lipase is the quantity of the enzyme that releases 1 µmole of fatty acids in 1 h at pH 7.2 at 37°C which is expressed as U/mL.[28,29]

Proteolytic Activity

Preparation of Substrate Solution

Two hundred millilitres of boiled milk was treated with acetic acid till casein precipitates out. The precipitates were then removed, dried and powdered. One gram of prepared casein was then diluted into 100 mL distilled water.

Procedure

One millilitre of substrate solution was taken in test tube followed by addition of 1 mL of 0.1M phosphate buffer (pH 7.6) and 1 mL calcium chloride. To this, 1 mL test enzyme solution was added to initiate protein digestion. It was then stopped after 1 h of incubation by adding 3 mL of 5% trichloroacetic acid solution. After 10 minutes precipitates were removed by centrifugation and one portion of supernatant was mixed with 5 mL Lowry's reagent. The mixture was then stained with diluted Folin-Ciocalteau reagent (1:2) and optical density measured at a wavelength of 650 nm. The proteolytic activity was calculated from standard curve. A unit of proteolyitc activity is the quantity of µmol of liberated tyrosine per mL of enzyme at 37°C which is expressed as U/mL of enzyme.[30]

Animal Groupings

The selected animals were divided in to three groups and each group consisted of six animals.

- Group-I (NC): Served as normal control and received distilled water as vehicle
- Group-II (DC): Served as disease control and received aspirin (200 mg/kg/day, p.o.)
- Group-III (TD): Served as test drug (i.e., Pep-Up Syrup) treated group and received Pep-Up Syrup (3 mL/kg/day, p.o.) + aspirin (200 mg/kg/day, p.o.).

Aspirin-induced Acute Gastric Ulcer Model in Rats

The selected animals were divided in to three groups as mentioned above. Test drug and aspirin was administered orally and repeated every 24 h for 7 days. Thirty minutes of interval was maintained between administration of test drug and aspirin in Group III. On the 8th day, animals in each group were fasted for 18 h after their assigned treatment. Animals were sacrificed with over anaesthesia and abdomen was opened by midline incision. Stomach was removed and cut along the greater curvature, washed with normal saline and stretched on paraffin bed. The glandular part was observed for ulceration and ulcer index[31] was determined. The samples of stomach tissue were analyzed to determine gastric wall mucus content,[32] lipid peroxidation (MDA),[33] reduced glutathione,[34] superoxide dismutase[35] and catalase.[36]

Histopathology of Stomach

Stomach from each animal was removed after sacrificing the animal under anaesthesia. It was collected in 10% formalin solution and immediately processed by paraffin technique. Section of approximately 5-µm thickness was cut and stained by haematoxylin and eosin (H and E). Sections were examined under microscope to evaluate structural changes.

Statistical Analysis

The different *in-vitro* experiments related to digestive property of Pep-Up Syrup were calculated in triplicate. The data was presented in Mean ± Standard Error of Mean (SEM). Results from aspirin-induced acute gastric ulcer model were also calculated using Mean ± SEM. Different groups were compared with analysis of variance (ANOVA) followed by *post hoc* Bonferroni's test. A $P < 0.05$ was considered as statistically significant.

RESULTS

In-vitro Study for Digestive Property of Pep-Up Syrup

The *in-vitro* digestive property was determined by means of evaluating amylolytic, lipolytic and proteolytic activity in comparison with blank. The amylolytic activity involves the breakdown of starch into maltose by the action of amylase enzyme. The amylolytic activity was found 0.250 ± 0.02 and 0.382 ± 0.02 in Pep-Up Syrup and amylase enzyme, respectively [Figure 1]. Lipolytic activity is another enzymatic activity that involves the breakdown of lipids into fatty acids by the action of lipase enzyme. The lipolytic activity was found 0.241 ± 0.02 and 0.613 ± 0.03 in Pep-Up Syrup and lipase enzyme, respectively [Figure 2]. Proteolytic activity is an enzymatic activity that involves breakdown of proteins into amino acids by the action of protease enzyme. The proteolytic activity was found 0.212 ± 0.02 and 0.419 ± 0.02 in Pep-Up Syrup and protease enzyme, respectively [Figure 3].

Effect of Pep-Up Syrup on Aspirin-Induced Acute Gastric Ulcer

The severity of aspirin-induced ulceration was found significantly ($P < 0.05$) decreased by Pep-Up Syrup in comparison of disease control group [Table 1]. Gastric wall mucus content was also found to be significantly decreased due to induction of aspirin. Pre-treatment of Pep-Up Syrup showed significant ($P < 0.05$) protection against loss occurred in gastric wall mucus content due to induction of aspirin [Table 1].

Antioxidant parameters like reduced glutathione, superoxide dismutase and catalase enzyme activity of stomach was significantly ($P < 0.05$) reduced due to induction of aspirin. Pep-Up Syrup treated group showed significant ($P < 0.05$) increase in all antioxidant parameters when compared with diseases control group [Table 2].

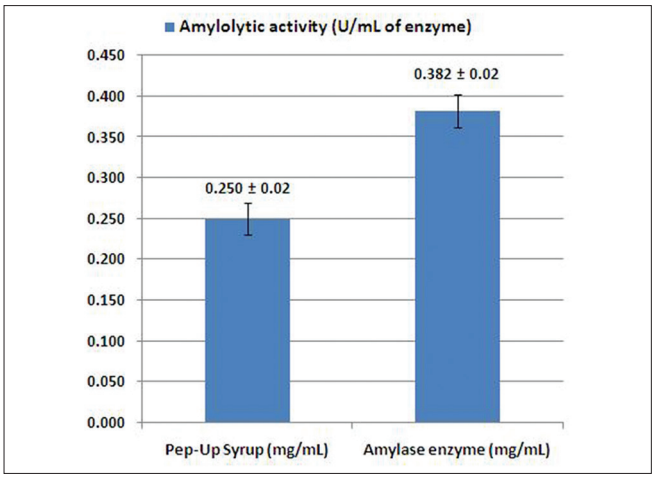

Figure 1: Amylolytic activity of Pep-Up Syrup

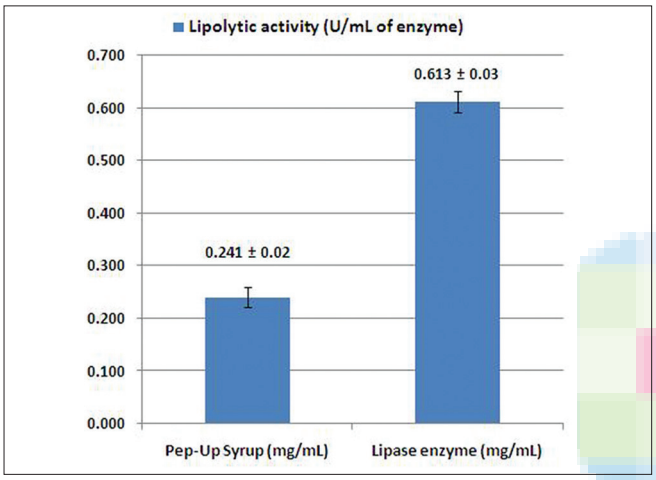

Figure 2: Lipolytic activity of Pep-Up Syrup

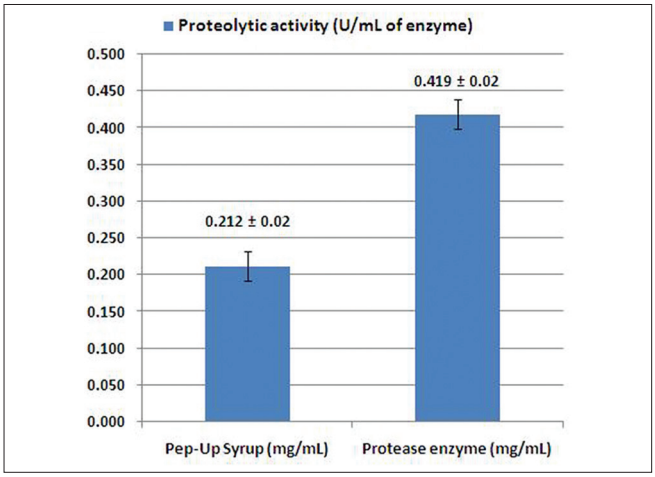

Figure 3: Proteolytic activity of Pep-Up Syrup

Aspirin caused significant ($P < 0.05$) increase in lipid peroxidation level in comparison of normal control group. Pep-Up Syrup treated group was significantly ($P < 0.05$) reduce lipid peroxidation level in comparison to disease control group [Table 3].

Table 1: Effect of Pep-Up Syrup on ulcer index and gastric wall mucus content in aspirin-induced gastric ulcer in rats

Group	Ulcer index	Gastric wall mucus content (µg/mL)
Normal control	0.00±0.00	423.09±34.56
Disease control	0.68±0.06#	200.98±21.00#
Pep-Up Syrup treated	0.47±0.04*	383.61±36.88*

All the values are expressed as mean±SEM (n=6) in each group, *P<0.05 as compared to disease control group; #P<0.05 as compared to normal control group

Table 2: Effect of Pep-Up Syrup on antioxidant biochemical parameters of stomach in aspirin-induced gastric ulcer in rats

Group	Reduced glutathione (µg/g of tissue)	Superoxide dismutase (µg/g of tissue)	Catalase enzyme activity (µmole H_2O_2 consumed/ min/g of tissue)
Normal control	882.55±41.97	239.72±19.97	770.47±49.56
Disease control	504.12±25.86#	100.25±12.86#	384.59±45.95#
Pep-Up Syrup treated	711.47±38.50*	197.88±12.86*	696.83±62.52*

All the values are expressed as mean ± SEM (n=6) in each group, *P<0.05 as compared to disease control group; #P<0.05 as compared to normal control group

Table 3: Effect of Pep-Up Syrup on lipid peroxidation level in aspirin-induced gastric ulcer in rats

Group	Lipid peroxidation (n mole/g of tissue)
Normal control	84.96±4.25
Disease control	173.08±10.84#
Pep-Up Syrup treated	119.96±7.67*

All the values are expressed as mean ± SEM (n=6) in each group, *P < 0.05 as compared to disease control group; #P < 0.05 as compared to normal control group.

Histopathological Findings

Histopathology of stomach of normal control rat showed normal cytoarchitecture of cells, oedema was not observed [Figure 4a]. Aspirin induction caused mucosal congestion, oedema and necrosis [Figure 4b]. Pep-Up Syrup treated group showed mild oedema and mucosal damage [Figure 4c].

DISCUSSION

Indigestion and gastric ulcer is the common conditions of GI tract. Ulcers are produced because of the inequity between aggressive and protective factor of the mucosal layer. To maintain the imbalance between aggressive and protective factor plenty of therapeutic agents are available. Most of these agents produce several adverse effects such as gynecomastia, acute interstitial nephritis, thrombocytopenia, nephrotoxicity and hepatotoxicity.[37-39] Ayurvedic formulations are a source of new drug and have

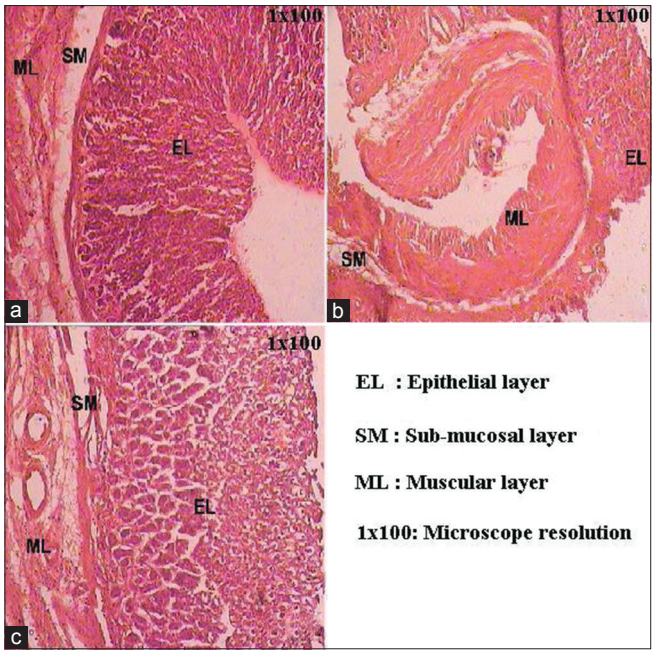

Figure 4: Histopathological pictures of stomach. (a) Normal control showing normal cytoarchitecture; (b) Disease control showing mucosal congestion, oedema and necrosis; (c) Pep-Up Syrup treated showing mild oedema and mucosal damage

been used frequently to treat indigestion and gastric ulcer. Hence, the present study was undertaken to investigate *in-vitro* digestive property and *in-vivo* anti-ulcer activity of Pep-Up Syrup in aspirin-induced acute gastric ulcer.

The *in-vitro* digestive property of Pep-Up Syrup was assessed by means of evaluating amylolytic, lipolytic and proteolytic activity in comparison with the standard enzyme. Pep-Up Syrup showed 0.250 ± 0.02, 0.241 ± 0.02 and 0.212 ± 0.02 amylolytic, lipolytic and proteolytic activity, respectively. This indicates comparable digestive property of Pep-Up Syrup with standard.

Synthetic non-steroidal anti-inflammatory (NSAIDs) like aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and blocking diffusion of H^+ diffusion resulting in damage to gastric mucosal barrier.[40,41] Administration of aspirin developed gastric ulceration and decreased content of gastric wall mucus. Pretreatment of Pep-Up Syrup provided significant protection against aspirin-induced gastric ulcer and mucosal damage. Free radicals has important role in the pathogenesis of the injury of various tissues, including the digestive system. Oxygen and hydrogen-derived free radicals like hydrogen peroxide, hydroxyl radicals can be the key elements for mucosal damage induced by non-steroidal anti-inflammatory drugs.[42] Treatment of Pep-Up Syrup significantly decreased lipid peroxidation level of stomach tissue which indicates effect of drug against free radicals damage. Antioxidant defensive system plays vital role against damage that occurs due to free radicals. It converts free radicals into non-toxic compounds.[17] In the present study, disease control rats showed significant decrease in superoxide dismutase, reduced glutathione levels and catalase enzyme activity when compared with normal control, indicating a dysfunction in antioxidant defensive system. Treatment with Pep-Up Syrup showed significant increase in level of antioxidant enzymes in comparison of disease control group. An antioxidant property of Pep-Up Syrup may be due to presence of *Withania somnifera* (Ashwagandha) Root,[9] *Zingiber officinale* (Shunthi) Rhizome,[17,18] and *Piper longum* (Pippali) Fruit.[19,20] All these ingredients have been well reported for having antioxidant property. Histopathology also indicates that the pretreatment of Pep-Up Syrup provides significant cyto-protection against aspirin-induced mucosal damage.

On the basis of study data it can be concluded that Pep-Up Syrup possesses the property of digesting starch, lipids and proteins. Data also revealed that Pep-Up Syrup has antiulcer property against aspirin-induced acute gastric ulcer.

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