ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.7826]

Journal Homepage: www.eijppr.com

Research Article

Evaluation of Pep-Up Tablet for *In-Vitro* Digestive Property and *In-Vivo* Anti-ulcer Activity

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Article info

Article History:
Received 29 November 2013
Accepted 31 December 2013

Keywords:
Pep-Up Tablet, *In-vitro* digestive property, *In-vivo* anti-ulcer activity, Anti-oxidant

Abstract

The objective of present research work was to investigate *in-vitro* digestive property and *in-vivo* anti-ulcer activity of Pep-Up Tablet in aspirin-induced acute gastric ulcer. *In-vitro* investigation of Pep-Up Tablet for digestive property was performed by means of evaluating amylolytic, lipolytic and proteolytic activity in comparison with blank. For *In-vivo* evaluation, on aspirin induced acute gastric ulcer model in rats, 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 Tablet treated respectively. Pep-Up Tablet (200 mg/kg/day) treatment was provided for 7 days orally. Ulcer index, gastric wall mucus content, lipid peroxidation level in stomach tissue and tissue anti-oxidant parameters like superoxide dismutase (SOD), reduced glutathione and catalase enzyme activity were carried out. Histopathology of stomach tissue was also performed. Statistical calculations were done by analysis of variance (ANOVA) followed by *post hoc* Bonferroni's test, with significant level of $p<0.05$. In present study, Pep-Up Tablet showed noticeable amylolytic, lipolytic and proteolytic activity. Pre-treatment of Pep-Up Tablet showed significant protection against aspirin induced ulceration and loss of gastric wall mucus content. Study also revealed significant increase in tissue anti-oxidant parameters. Histopathology study revealed that Pep-Up Tablet provides significant cyto-protection against aspirin induced mucosal damage. On the basis of study data, it can be concluded that Pep-Up Tablet possesses considerable property of digesting starch, lipids and proteins. Data also revealed that Pep-Up Tablet exhibits anti-ulcer activity against aspirin induced acute gastric ulcer.

1. INTRODUCTION

Gastrointestinal (GI) problems are the most common and widespread health complaints among the general populace. Amongst them Indigestion (Dyspepsia) is a very common primary care concern.[1] The term Indigestion (Dyspepsia) refers to a cluster of symptoms thought to arise in the upper gastrointestinal tract. It comprises symptoms such as epigastric pain, regurgitation, heartburn, belching, nausea, vomiting, and early satiety.[2] 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).[3] 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.[4] Surveys in Western societies have recorded a prevalence of this condition between 23 and 41%. The specific record of prevalence in the U.S. is 26% and 41% in the U.K. Although only 20 to 25% of persons with dyspepsia seek medical care only 2 to 5% of problems reaches to primary care physicians.[5] In India, 49.40% population is suffering from digestive problems amongst them 21.10% is aging between 12-20 years and 76.20% is aging above 70 years.[6]

Peptic ulcer (gastric ulcer) is deep lesion penetrating through the entire thickness of the gastro intestinal tract mucosa and muscular layer. The exact cause of peptic ulcer is not known, but it may occur 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.[7] In Ayurveda, peptic ulcers or gastro-duodenal ulcers generally refer to *Parinamasula*.[8] Peptic ulcer is worldwide problem and its prevalence is quite high in India. Several field studies from different parts of our country suggest its occurrence in 3 to 10 per thousand populations.[9]

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.[10]

Pep-Up Tablet is such an Ayurvedic formulation which contains extracts of *Emblica officinalis* (Amalaki) Fruit[11-13], *Terminalia chebula* (Haritaki) Fruit[14,15], *Plumbago zeylanica* (Chitrak) Root[16,17], *Trachyspermum ammi* (Ajwain) Fruit[18,19] and powders of Sodii carbonas (Swarjikakshar) Mineral[20], *Zingiber officinale* (Shunthi) Rhizome[21,22], Rock salt (Saindhav) Mineral[23], Black salt (Kala namak) Mineral[24], *Piper nigrum* (Kali mirch) Fruit[25], *Piper longum* (Pippali) Fruit[26,27]. It is a proprietary Ayurvedic medicine manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. Majority of ingredients of Pep-Up Tablet 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 their combination.

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

2. MATERIALS AND METHODS

2.1 Experimental Animals

Wistar albino rats of 200-250 g were used and acclimatized 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.

2.2 Administration of Test Drug and Dosage

The test drug (i.e. Pep-Up Tablet) was received from Vasu Healthcare Pvt. Ltd., Baroda, Gujarat, India. It was administered orally as suspension by triturating with vehicle 1% sodium carboxy methyl cellulose (Na-CMC). 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.[28]

2.3 Determination of I*n-vitro* Digestive Property

100 mg Pep-Up Tablet powder was extracted 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.

2.3.1 Amylolytic Activity

1 mL of starch solution (soluble starch1% 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 in UV spectrophotometer at 520 nm. A unit of amylase is expressed as U/mL of maltose produced during 5 min. incubation with 1% starch solution.[29]

2.3.2 Lipolytic Activity

Preparation of substrate solution:

2 mL of castor oil was, neutralized 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:

20 mL 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^{o}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 total reaction 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 1µmole of fatty acids in 1 h at pH 7.2 at 37°C which is expressed as U/mL.[30,31]

2.3.3 Proteolytic Activity

Preparation of substrate solution:

200 mL of boiled milk was treated with acetic acid till casein precipitates out. The precipitates were then removed, dried and powdered. 1 g of prepared casein was then diluted into 100 mL distilled water.

Procedure:

1 mL 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.[32]

2.4 Animal Groupings

The selected animals were divided in to three groups where 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 Tablet) treated group and received Pep-Up Tablet (200 mg/kg/day, p.o.) + aspirin (200 mg/kg/day, p.o.)

2.5 Aspirin Induced Acute Gastric Ulcer Model in Rats[33,34]

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. 30 min 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 anesthesia 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[33] was determined. The samples of stomach tissue were analyzed to determine gastric wall mucus content[34], lipid peroxidation (MDA)[35], reduced glutathione[36], superoxide dismutase[37] and catalase[38].

2.6 Histopathology of Stomach

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

2.7 Statistical Analysis

The different *in-vitro* experiments related to digestive property of Pep-Up Tablet 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.

3. RESULTS

3.1 In-vitro Study for Digestive Property of Pep-Up Tablet

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 of Pep-Up Tablet and standard amylase enzyme was found 0.179 ± 0.02 and 0.382 ± 0.02 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 of Pep-Up Tablet and standard lipase enzyme was found 0.200 ± 0.02 and 0.613 ± 0.03 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 of Pep-Up Tablet was found 0.153 ± 0.02 and 0.419 ± 0.02 with standard protease enzyme (Figure 3).

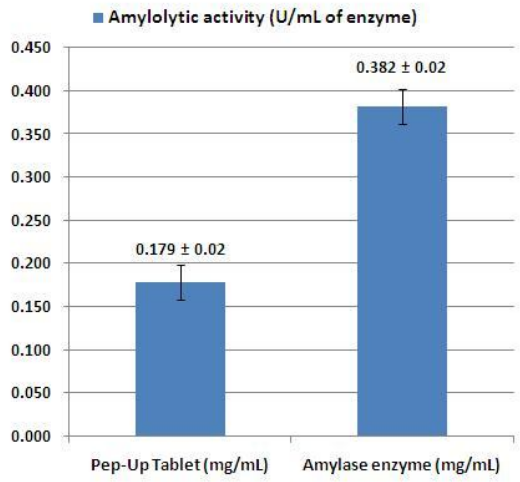

Figure 1: Amylolytic activity of Pep-Up Tablet

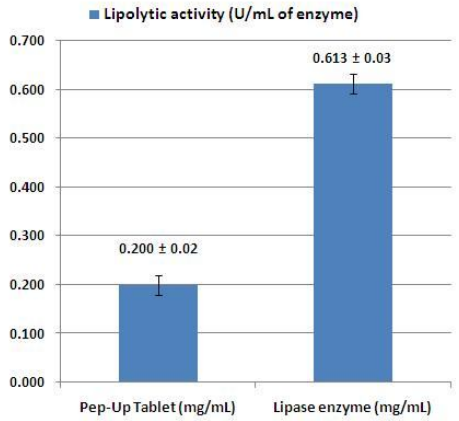

Figure 2: Lipolytic activity of Pep-Up Tablet

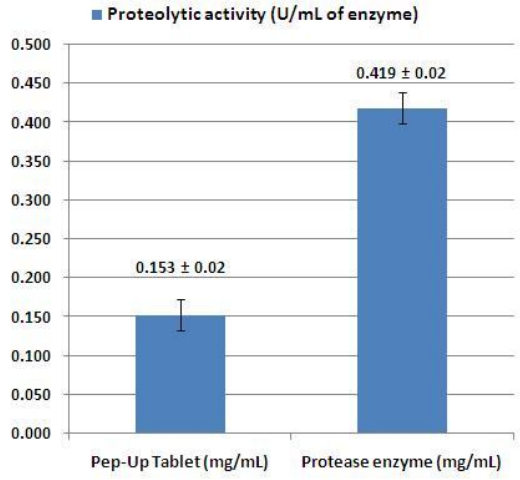

Figure 3: Proteolytic activity of Pep-Up Tablet

3.2 Effect of Pep-Up Tablet on Aspirin Induced Acute Gastric Ulcer

The severity of aspirin induced ulceration was found significantly ($p<0.05$) decreased by Pep-Up Tablet in comparison of disease control group (Table 1). Gastric wall mucus content was observed to be significantly decreased on induction of aspirin. Pre-treatment of Pep-Up Tablet showed significant ($p<0.05$) protection against aspirin induced loss of gastric wall mucus content (Table 1).

Table 1: Effect of Pep-Up Tablet 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 Tablet treated	0.45 ± 0.04*	388.16 ± 36.31*

All the values are expressed as mean ± SEM (n=6) in each group. $^{*}p<0.05$ when compared to disease control group. $^{\#}p<0.05$ when compared to normal control group.

Induction of aspirin significantly reduced ($p<0.05$) anti-oxidant parameters like reduced glutathione, superoxide dismutase and catalase enzyme activity of stomach. Pep-Up Tablet treated group showed significant ($p<0.05$) increase in all anti-oxidant parameters when compared with diseases control group. (Table 2).

Table 2: Effect of Pep-Up Tablet on anti-oxidant 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 Tablet treated	704.61 ± 40.66*	190.91 ± 15.28*	664.11 ± 61.26*

All the values are expressed as mean ± SEM (n=6) in each group. $^{*}p<0.05$ when compared to disease control group. $^{\#}p<0.05$ when compared to normal control group.

Aspirin caused significant ($p<0.05$) increase in lipid peroxidation level in comparison of normal control group. Pep-Up Tablet treated group significantly ($p<0.05$) reduced lipid peroxidation level in comparison to disease control group. (Table 3)

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

Group	Lipid peroxidation (nmole/g of tissue)
Normal control	84.96 ± 4.25
Disease control	173.08 ± 10.84$^{\#}$
Pep-Up Tablet treated	127.88 ± 8.79*

All the values are expressed as mean ± SEM (n=6) in each group. $^{*}p<0.05$ when compared to disease control group. $^{\#}p<0.05$ when compared to normal control group.

3.3 Histopathological Findings

Histopathology of stomach of normal control rat showed normal cyto-architecture of cells and no edema was observed (Figure 4 (a)). Stomach tissue of aspirin induced rats exhibited disrupted mucosal layer, edema and necrosis (Figure 4 (b)). Pep-Up Tablet treated group showed mild edema and mucosal damage as compared to disease control (Figure 4 (c)).

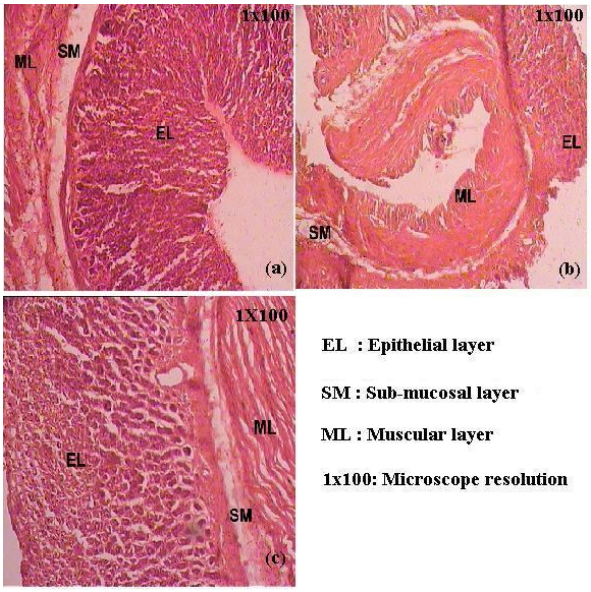

Figure 4: Histopathological pictures of stomach. (a) Normal control showing normal cyto-architecture; (b) Disease control showing mucosal disruption, edema and necrosis; (c) Pep-Up Tablet treated showing mild edema and mucosal damage as compared to disease control.

4. DISCUSSION

Indigestion and gastric ulcer is the common conditions of gastrointestinal 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.[39-41] Ayurvedic formulations are a source of new drug and have been generally believed to be safe 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 Tablet in aspirin-induced acute gastric ulcer.

The *in-vitro* digestive property of Pep-Up Tablet was assessed by means of evaluating amylolytic, lipolytic and proteolytic activity in comparison with the standard enzyme. Pep-Up Tablet showed comparable amylolytic, lipolytic and proteolytic activity against standard enzymes (Figure 1 to 3).

Synthetic non-steroidal anti-inflammatory (NSAIDs) like aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and blocking of H^+ diffusion resulting in damage to gastric mucosal barrier.[42,43] Administration of aspirin developed gastric ulceration and decreased content of gastric wall mucus (Table 1). Pretreatment of Pep-Up Tablet provided significant gastro protection against aspirin induced gastric ulcer and mucosal damage.

In the pathogenesis of various tissue injury of the digestive system, free radicals also have important role. Oxygen and hydrogen derived free radicals like hydrogen peroxide, hydroxyl radicals can be responsible for mucosal damage. These are majorly induced by non-steroidal anti-inflammatory drugs.[44] Treatment of Pep-Up Tablet significantly decreased lipid peroxidation level of stomach tissue which indicates significant effect of drug against free radicals damage (Table 3). Anti-oxidant defensive system also plays vital role in recovery of damage that occurs due to free radicals. It converts free radicals into non-toxic compounds.[21] In the present study, disease control rats showed significant decrease in superoxide dismutase, reduced glutathione levels and catalase activity when compared with normal control, indicating a dysfunction in anti-oxidant defensive system. Treatment with Pep-Up Tablet showed significant increase in level of anti-oxidant enzymes in comparison of disease control group (Table 2). An anti-oxidant property of Pep-Up Tablet may be due to presence of *Emblica officinalis* (Amalaki) Fruit[11-13], *Terminalia chebula* (Haritaki) Fruit[14,15], *Zingiber officinale* (Shunthi) Rhizome[21,22], *Piper nigrum* (Kalimirch) Fruit[25] and *Piper longum* (Pippali) Fruit[26,27]. All these ingredients have been well reported for having anti-oxidant property. Histopathology also indicates that pretreatment of Pep-Up Tablet provides significant cyto-protection against aspirin induced mucosal damage.

5. CONCLUSION

On the basis of study data, it can be concluded that Pep-Up Tablet possesses the property of digesting starch, lipids and proteins. Data also revealed that Pep-Up Tablet exhibits anti-ulcer activity in aspirin induced acute gastric ulcer.

6. ACKNOWLEDGEMENT

Authors are sincerely thankful to the management of Vasu Healthcare Pvt. Ltd. for providing test drug samples and Babaria Institute of Pharmacy for providing the necessary facilities for conducting the study.

7. SOURCE OF SUPPORT: Nil

8. CONFLICTS OF INTERESTS:

The author(s) have no competing interests for finance, publication of this research, patents and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript.

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