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Gastroprotective and anti-secretory effect of Pep-Up Tablet on pyloric ligation-induced gastric ulcer model in rats

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ABSTRACT

Aim of the study was to investigate Gastroprotective and anti-secretory effect of Pep-Up Tablet on pyloric ligation-induced gastric ulcer model in rats. Pep-Up Tablet is an Ayurvedic proprietary formulation which is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. The selected animals were divided into two groups and each group consisted of six animals. Group-I was considered as disease control and Group-II was as test drug (Pep-Up Tablet) treated group. Pep-Up Tablet (200 mg/kg/day, p.o.) was administered for 7 days by oral route in Group-II. Pep-Up Tablet was studied for its effect on ulcer index, gastric wall secretory parameters and mucin activity. Pretreatment of Pep-Up Tablet showed significant reduction in ulcer index, gastric acid secretion and pepsin activity. Pep-Up Tablet significantly increased mucin activity (TC:TP ratio) as well which was due to significant increase in the total carbohydrate content. From the available data of present study, it can be concluded that Pep-Up Tablet plays important role mainly on inhibition of acid secretion and in increase of mucin secretion which in turn enhances the stability of gastric mucosal barrier. Pep-Up Tablet provided significant gastric cytoprotection against pyloric ligation-induced gastric ulceration in rats.

INTRODUCTION

Peptic ulcer is assumed to develop when the summation of aggressive factors (like acid, pepsin and *Helicobacter pylori* infection) is greater than defensive factors (like secretion of bicarbonate, mucus and prostaglandins) (Jain *et al.*, 2007). It is worldwide problem and its prevalence is quite high in India. Several field studies from different parts of our country suggest its occurrence in 4 to 10 per thousand populations. Three states of India viz. Tamil Nadu, Andhra Pradesh, and Jammu and Kashmir are considered to be very high risk areas (Khushtar *et al.*, 2009). The exact cause of peptic ulcer is not known, the disease results in chronic suffering, loss of working hours and occasional fatality. Smoking, alcoholism, and spices add to the severity of the disease that often precipitate serious complication of ulcer (Khushtar *et al.*, 2009). Over the past few decades, there has been surge in research activity aimed towards the development of effective and safe antiulcer drugs both synthetically and from natural resources.

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Considering the safety aspect, indigenous drugs possessing more safe should be looked for as a better alternative for the treatment of peptic ulcer (Bafna and Balaraman, 2005). In Indian System of Medicine, several plants have been used to treat gastrointestinal disorders, including gastric ulcers (Langmead and Rampton, 2006) and the identical chemical compound of these plants are also reported for gastroprotective activity (Gadekar *et al.*, 2010). Pep-Up Tablet is such an Ayurvedic formulation which contains extracts of *Emblica officinalis* (Amalaki) Fruit (Rao and Siddiqui, 1964, Singh and Sharma, 1971, Al-Rehaily *et al.*, 2002), *Terminalia chebula* (Haritaki) Fruit (Suryaprakash *et al.*, 2012, Raju *et al.*, 2009), *Plumbago zeylanica* (Chitrak) Root (Falang *et al.*, 2012, Datta and Mishra, 2012), *Trachyspermum ammi* (Ajwain) Fruit (Bairwa *et al.*, 2012, Ramaswamy *et al.*, 2010) and powders of Sodii carbonas (Swarjikakshar) Mineral (Pandey and Chunekar, 2002), *Zingiber officinale* (Shunthi) Rhizome (Wang *et al.*, 2011, Goel and Sairam, 2002), Rock salt (Saindhav) Mineral (Pandey and Chunekar, 2002), Black salt (Kala namak) Mineral (Pandey and Chunekar, 2002), *Piper nigrum* (Kali mirch) Fruit (Boddupalli *et al.*, 2012), *Piper longum* (Pippali) Fruit (Manoj *et al.*, 2004, Agrawal *et al.*, 2000).

It is 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 Gastroprotective effect. However, no such evidence was found which proves efficacy of such combination. The present study was aimed to investigate gstroprotective and anti-secretory effect of Pep-Up Tablet on pyloric ligation-induced gastric ulcer model in rats.

MATERIALS AND METHODS

Experimental animals

Adult wistar albino rats of either sex weighing 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 Chakkan 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/12-13/11) 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 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 (Paget and Barnes, 1964). Experimental dose of Pep-Up Tablet was 200 mg/kg/day (p.o.).

Experimental design

The selected animals were divided into two groups and each group consisted of six animals. Group-I (DC), Disease control, received distilled water as vehicle and exposed to pyloric ligation. Group-II (TD), test drug treated group, received Pep-Up Tablet (200 mg/kg/day, p.o.) and exposed to pyloric ligation. Test drug was administered for 7 days by oral route in group-II. On 7^{th} day, one hour after test drug administration, pylorus was ligated (Shay *et al.*, 1945). The rats were housed in individual metabolic cages to prevent caprophagy and overnight fasted with access to water *ad libitum* one day prior to ligation. Rats were anesthetized with ether anesthesia and the portion of abdomen was opened in layer by a small midline incision just below and lateral to the xiphoid process. Pylorus portion of the stomach was slightly lifted out avoiding traction to the pylorus or damage to its blood supply. The pylorus was ligated with cotton thread and stomach was replaced carefully. The incision was closed with interrupted sutures in layers. The animals were deprived of both food and water during the post operative period and were sacrificed by an over dose of ether at the end of 19 hours after pyloric ligation. Abdominal cavity was reopened carefully and the stomach was excised after ligating the terminal portion of esophagus to prevent loss of gastric contents during excision. Gastric contents were drained into tubes and centrifuged at 3000 rpm for 15 min. Volume of gastric juice was noted and used for biochemical estimations. Stomach biopsies were taken for assessment of stomach ulcer.

Assessment of stomach damage

The stomach was excised, cleaned and opened along its greater curvature, and then the inner surface was gently washed with cold saline solution and examined for ulceration with a magnifying lens. Severity and total number of ulcers in each rat were recorded for calculating ulcer index (Suzuki *et al.*, 1976).

Gastric secretion

The volume of the gastric juice supernatant was expressed as mL per 100 g body weight of rats. Free and total acidity were estimated by titrimetric method using 0.1 N NaOH. Phenolphthalein was used as an indicator. Free acidity and total acidity were expressed as mEq HCl/L (Kulkarni, 2002). Pepsin activity was determined using hemoglobin as substrate (Sanyal *et al.*, 1983) and has been expressed as μmol of tyrosine/mL of gastric juice. Dissolved muco-substances were estimated in 95% alcoholic precipitates of the gastric juice. This precipitates were employed for estimation of total protein (Lowry *et al.*, 1951), total hexose (Dische and Shettles, 1948), hexosamine (Dische and Borenfreund, 1950), fucose (Winzler, 1958), and sialic acid (Warren, 1959). The result has been expressed as μg/mL of gastric juice. The ratio of total carbohydrates (TC) (sum of total hexose, hexosamine, fucose and sialic acid) to protein (TP) has been taken as index of mucin activity (TC:TP ratio) (Sanyal *et al.*, 1983).

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM) for six rats per experimental group. Unpaired '*t*' test was used to compare the mean values of quantitative variables among the groups.

RESULTS AND DISCUSSION

There are various methods for the production of peptic ulcers in animals where pyloric ligation is one of the most commonly used methods (Ghosh, 2005). Pyloric ligation causes accumulation of the gastric juice and hindrance of the gastric blood circulation, which lead to damage of the upper gastrointestinal tract and the formation of ulcers (Sachin and Archana, 2009). In present study pyloric ligation significantly elevated gastric acid secretion and ulcer score. Level of total hexose, hexosamine, total fucose, sialic acid, total carbohydrate (TC) and mucin activity (TC:TP ratio) in gastric juice were significantly decreased. The results of disease control group are supported by similar type of study (Nariya *et al.*, 2013).

Table 1: Effect of Pep-Up Tablet on ulcer index and gastric acid secretion.

Groups	Ulcer index	Volume of gastric juice (mL/100 g)	Free acidity (mEq HCl/L)	Total acidity (mEq HCl/L)	Pepsin activity (µmol of tyrosine/mL)
Disease control	6.23 ± 0.42	2.81 ± 0.23	44.84 ± 2.55	139.01 ± 8.72	816.71 ± 15.22
Pep-Up Tablet treated	3.91 ± 0.21**	1.48 ± 0.11**	35.30 ± 2.05**	60.18 ± 6.38***	511.04 ± 14.47**

All the values are expressed as mean ± SEM (n=6) in each group. **p<0.01 and ***p<0.001 as compared to disease control group.

Table 2: Effect of Pep-Up Tablet on level of total hexose, hexosamine, total fucose, sialic acid, total carbohydrate, total protein and mucin activity (TC:TP Ratio) in gastric juice.

Groups	Total hexose (µg/mL)	Hexosamine (µg/mL)	Total fucose (µg/mL)	Sialic acid (µg/mL)	Total carbohydrate (µg/mL)	Total protein (µg/mL)	TC:TP ratio
Disease control	258.98 ± 24.48	223.31 ± 22.23	17.06 ± 1.38	43.98 ± 2.95	543.34 ± 43.61	179.48 ± 16.21	3.23 ± 0.51
Pep-Up Tablet treated	395.02 ± 27.83*	328.97 ± 29.60**	27.26 ±2.15**	70.84 ± 4.92**	822.10 ± 57.99***	166.66 ± 18.38	5.05 ± 0.28**

All the values are expressed as mean ± SEM (n=6) in each group. *p<0.05, **p<0.01 and ***p<0.001 as compared to disease control group.

Pyloric ligation for 19 h caused gastric mucosal ulcers in rats was calculated as ulcer index (Table 1). Pretreatment of Pep-Up Tablet showed significant ($p < 0.01$) decrease in gastric ulcer index when compared to disease control (Table 1) which indicates gastric cytoprotective effect of Pep-Up Tablet.

Pretreatment of Pep-Up Tablet significantly reduced the free acidity ($p<0.01$) and total acidity ($p<0.001$) in comparison to disease control group (Table 1). Volume of gastric juice ($p<0.01$) and pepsin activity ($p<0.01$) were also significantly decreased by pretreatment of Pep-Up Tablet (Table 1). The data related to the concentration of individual carbohydrate constituents and mucin activity is presented in Table 2. The level of total hexose, hexosamine, total fucose and sialic acid were significantly increased in Pep-Up Tablet treated groups. Total carbohydrate values in gastric juice of the rats were significantly increased in Pep-Up Tablet ($p<0.001$) treated group. The significant increase in total carbohydrate was due to increase in mucopolysaccharides like hexose, hexosamine, fucose and Sialic acid leading to significant increase in mucin activity (TC:TP ratio).

It is reported that the medicinal properties of many plants are attributed mainly to the presence of flavonoids, coumarins, alkaloids, terpenoids, tannins, phenolic acids, and antioxidant micronutrients (Czinner *et al.*, 2001). Phenolic compounds occur as secondary metabolites and are widely distributed in plants from the roots to the seeds (Antolovich *et al.*, 2000). Ellagic and gallic are major phenolic acids found in *Emblica officinalis* and *Terminalia chebula*.

The potential of Pep-Up Tablet to reduce the acidity may be due to the direct action on the acid producing parietal cells or through inhibition of the proton pumping H^+-K^+ ATPase. As earlier study shows that ellagic acid appeared to contribute toward better H^+-K^+ ATPase inhibitory activity (Murakami *et al.*, 1991) while gallic acid contributed significantly to anti-oxidant activity (Gow-Chin *et al.*, 2002). Piperine is also reported for anti-oxidant and anti-ulcerogenic activity (Patra *et al.*, 2014, Mittal and Gupta, 2000) which is the major chemical constituent of *Piper nigrum* and *Piper longum.*

Pep-Up Tablet significantly increased mucin activity (TC:TP ratio), which reflects the functional integrity of mucosal barrier and could be taken as a reliable index for mucin secretion (Sanyal *et al.*, 1983). The increase in mucin activity was due to significant increase in the total carbohydrate content. Hence, the augmentation of the mucosal barrier by Pep-Up Tablet was due to increase in the secretion of dissolved mucus in the gastric secretion.

CONCLUSION

From the available data of present study, it can be concluded that Pep-Up Tablet plays important role in gastric protection mainly by inhibiting local acid secretion and by attaining increase in mucin secretion thereby enhancing the stability of gastric mucosal barrier. Pep-Up Tablet provides significant gastric cytoprotection against pyloric ligation-induced gastric ulceration in rats.

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REFERENCES

Agrawal AK, Rao CV, Sairam K, Joshi VK, Goel RK. Effect of *Piper longum* Linn, *Zingiber officinalis* Linn and Ferula species on gastric ulceration and secretion in rats. Indian J Exp Biol, 2000; 38(10): 994-998.

Al-Rehaily AJ, Al-Howiriny TA, Al-Sohaibani MO, Rafatullah S. Gastroprotective effects of 'Amla' *Emblica officinalis* on in vivo test models in rats. Phytomedicine, 2002; 9(6): 515-522.

Antolovich M, Prenzler P, Robards K, Ryan D. Sample preparation in the determination of phenolic compounds in fruits. Analyst, 2000; 125: 989-1009.

Bafna PA, Balaraman R. Anti-ulcer and antioxidants activity of Pepticare, a herbo-mineral formulation. Phytomedicine, 2005; 12: 264-270.

Bairwa R, Sodha RS, Rajawat BS. *Trachyspermum ammi*. Pharmacogn Rev, 2012; 6(11): 56-60.

Boddupalli B, Ramani R, Subramaniam B, Anisetti R. *In-vitro* and *in-vivo* evaluation of hepato-protection and anti-ulcer activities of piperine gastro retentive micropspheres. Asian Pac J Trop Biomed, 2012; S1237-S1240.

Czinner E, Hagymasi K, Blazovics A, Kery A, Szoke E, Lemberkovics E: The *in vitro* effect of *Helichysi flos* on microsomal lipid peroxidation. J Ethnopharmacol 2001;77:31-5.

Datta S, Mishra R. *Plumbago zeylanica* Linn. (Chitrak) - Review as Rasayan (Rejuvenator/Antiaging). Int J Res Pharm Biomed Sci, 2012; 3(1): 250-267.

Dische Z, Borenfreund E. A spectrophotometric method for the micro determination of hexosamines. J Biol Chem, 1950; 184: 517-522.

Dische Z, Shettles LB. A specific colour reaction of methylpentoses and a spectrophotometric micromethod for their determination. J Biol Chem, 1948; 175: 595-603.

Falang KD, Uguru MO, Wannang NN, Azi HH, Chiamaka N. Anti-ulcer activity of *Plumbago zeylanica* Linn. root extract. J Nat Prod Plant Resour, 2012; 2(5): 563-567.

Gadekar R, Singour PK, Chaurasiya PK, Pawar RS, Patil UK. A potential of some medicinal plants as an antiulcer agents. Pharmacogn Rev, 2010; 4: 136-146.

Ghosh MN. 2005. Fundamentals of experimental pharmacology. 3rd ed. Kolkata: Hilton & Company.

Goel RK, Sairam K. Anti-ulcer drugs from indigenous sources with emphasis on *Musa sapientum*, tamra bhasma, *Asparagus racemosus* and *Zingiber officinale*. Indian J Pharmacol, 2002; 34: 100-110.

Gow-Chin Yen, Pin-Der Duh, Hui-Ling Tsai. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem, 2002; 79(3): 307-313.

Jain KS, Shah AK, Bariwal J, Shelke SM, Kale AP, Jagtap JR, Bhosale AV. Recent advances in proton pump inhibitors and management of acid-peptic disorders. Bioorg Med Chem, 2007; 15: 1181-1205.

Khushtar M, Kumar V, Javed K, Bhandari U. Protective effect of Ginger oil on aspirin and pylorus ligation-induced gastric ulcer model in rats. Indian J Pharm Sci, 2009; 71(5): 554-558.

Kulkarni SK. 2002. Handbook of Experimental Pharmacology. 3rd ed. Delhi: Vallabh Prakashan.

Langmead L, Rampton DS. Complementary and alternative therapies for inflammatory bowel disease. Aliment Pharmacol Ther, 2006; 23: 341-349.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem, 1951; 193: 265-275.

Manoj P, Soniya EV, Banerjee NS, Ravichandran P. Recent studies on well-known spice, *Piper longum* Linn. Nat Prod Rad, 2004; 3(4): 222-227.

Mittal R, Gupta RL. In vitro antioxidant activity of piperine. Methods Find Exp Clin Pharmacol, 2000; 22(5): 271-274.

Murakami S, Isobe Y, Kijima H, Nagai H, Muramatu M, Otome S. Inhibition of gastric H^+-K^+ ATPase and acid secretion by ellagic acid. Planta Medica, 1991; 57: 305-308.

Nariya M, Tomar R, Shukla V, Ravishankar B, Jain S. The inhibition of gastric mucosal injury by Chinnodbhavadi kwath (decoction) – an Indian Ayurvedic Formulation in rats. J Exp Integr Med, 2013; 3(3): 243-247.

Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. Pharmacometrics. Vol. I. New York: Academic Press; 1964, 161.

Pandey GS, Chunekar KC. 2002. Bhavprakash Nighantu of shri bhavamisra. Published by chaukhambha bharati academy, Varanasi, India.

Patra KC, Jayaram Kumar K, Ahirwar DK. Gastroprotective effect of standardized extract of *Amukkara choornam* on experimental gastric ulcer in rats. J Nat Med, 2014; 68(2): 284-294.

Raju D, Ilango K, Chitra V, Ashish K. Evaluation of Anti-ulcer activity of methanolic extract of *Terminalia chebula* fruits in experimental rats. J Pharm Sci Res, 2009; 1(3): 101-107.

Ramaswamy S, Sengottuvelu S, Sherief S, Jaikumar S, Saravanan R, Prasadkumar C, Sivakumar T. Gastroprotective activity of ethanolic extract of *Trachyspermum ammi* fruit. Int J Pharm Biosci, 2010; 1(1): 1-15.

Rao M, Siddiqui H. Pharmacological studies on *Emblica officinalis* Gaertn. Indian J Exp Biol, 1964; 2: 29-31.

Sachin SS, Archana RJ. Antiulcer activity of methanol extract of *Erythrina indica* Lam. leaves in experimental animals. Phcog Res, 2009; 1: 396-401.

Sanyal AK, Mitra PK, Goel RK. A modification method to estimate dissolved mucosubstances in gastric juice. Ind J Exp Biol, 1983; 21: 78-80.

Shay H, Komarov SA, Fels SE, Meraze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in rat. Gastroenterology, 1945; 5: 43-61.

Singh B, Sharma P. Effect of Amalaki on amalapitta. J Res Ind Med, 1971; 5: 223-229.

Suryaprakash DV, Sreesatya N, Avanigadda S, Vangalapati M. Pharmacological Review on *Terminalia chebula*. Int J Res Pharm Biomed Sci, 2012; 3(2): 679-683.

Suzuki Y, Hayashi M, Ito M, Yamagami J. Antiulcer effect of 4'-(2-carboethyl) phenyltrans-4-amino methyl cyclohexane carboxylate hydrochloride (Cetraxate) on various experimental ulcers in rats. Japanese J Pharmacol, 1976; 26: 471-480.

Wang Z, Hasegawa J, Wang X, Matsuda A, Tokuda T, Miura N, Watanabe T. Protective effects of Ginger against aspirin-induced gastric ulcers in rats. Yonago Acta Med, 2011; 54(1): 11-19.

Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem, 1959; 234: 1971-1975.

Winzler RJ. 1958. Determination of serum glycoprotein. In: Methods of Biochemical analysis. Glick E (eds.), Vol. II. Inter science Publishers Limited, London.

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