International Journal of Herbal Medicine
Available online at www.florajournal.com

IJHM
International Journal of Herbal Medicine

ISSN 2321-2187
IJHM 2014; 2 (2): 27-30
Received: 19-04-2014
Accepted: 08-05-2014

Hardik Soni
Vasu Research Centre, A Division of Vasu Healthcare Pvt. Ltd., 896/A, G.I.D.C., Makarpura, Vadodara – 390 010, Gujarat, India

Sweta Patel
Department of Pharmacology, Babaria Institute of Pharmacy, BIT education campus, Vadodara-Mumbai NH#8, Varnama , Vadodara – 391 240, Gujarat, India

Arindam Paul
Department of Pharmacology, Babaria Institute of Pharmacy, BIT education campus, Vadodara-Mumbai NH#8, Varnama , Vadodara – 391 240, Gujarat, India

Ghanshyam Patel
Vasu Research Centre, A Division of Vasu Healthcare Pvt. Ltd., 896/A, G.I.D.C., Makarpura, Vadodara – 390 010, Gujarat, India.

Correspondence:
Hardik Soni
Asst. Manager, R&D Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.) 896/A, G.I.D.C., Makarpura, Vadodara-390010, Gujarat, India.

Evaluation of gastro-protective effect of Pep-Up Tablet and Pep-Up Syrup on water immersion plus restraint stress-induced gastric ulcer in rats

Hardik Soni, Sweta Patel, Arindam Paul, Ghanshyam Patel

ABSTRACT
Stress is an adaptive physiological response to disruption of homeostasis. Stress-induced gastric ulceration is a typical example of stress-associated organ injuries. The present study was carried out to evaluate gastroprotective effect of Pep-Up Tablet and Syrup, proprietary Ayurvedic formulations, on water immersion plus restraint stress-induced gastric ulcer in rats. Both test drugs were studied for their effect on ulcer index, gastric wall mucus content, lipid peroxidation level in stomach tissue and tissue anti-oxidant parameters like superoxide dismutase, reduced glutathione and catalase activity. Pre-treatment of Pep-Up Tablet and Syrup showed significant protection against loss of gastric wall mucus content and ulceration. They also significantly prevented depletion of tissue anti-oxidant parameters in comparison of disease control group. From the present study, it can be concluded that Pep-Up Tablet and Syrup are having significant anti-oxidant property which enhanced the stability of gastric mucosa against the damage caused by water immersion plus restraint induced stress. The gastro-protective effect of Pep-Up Tablet and Syrup on stress induced gastric ulceration supports their clinical application to prevent acid-peptic disorders.

Keywords: Pep-Up Tablet, Pep-Up Syrup, ulcer index, gastric wall mucus content, anti-oxidant property, gastro-protective effect

1. Introduction
Stress is an adaptive physiological response to disruption of body's internal homeostasis. Excessive stress can induce organ injury or contribute to diseases, such as gastric ulcers, hypertension, diabetes, and cancer. Stress-induced gastric ulceration is a typical example of stress-associated organ injuries [1]. Acute gastric mucosal injury is a serious clinical problem worldwide [2]. Since the development of gastric lesions during restraint stress was significantly enhanced by exposure to water immersion. The rise in acid secretion may be an important aggravating factor in the process of ulceration during water immersion [1].
The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [3]. Recently, it has also been reported that many traditional Asian herbal remedies act on gastro-intestinal diseases [4]. Pep-Up Tablet and Syrup are Ayurvedic proprietary formulations manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara.
Pep-Up Tablet contains extracts of *Emblica officinalis* (Amalaki) Fruit, [5-7] *Terminalia chebula* (Haritaki) Fruit, [8, 9] *Plumbago zeylanica* (Chitrak) Root, [10, 11] *Trachyspermum ammi* (Ajwain) Fruit [12, 13] and powders of Sodii carbonas (Swarjikakshar) Mineral, **[14]** *Zingiber officinale* (Shunthi) Rhizome, [15, 16] Rock salt (Saindhav) Mineral, [17] Black salt (Kala namak) Mineral, **[18]** *Piper nigrum* (Kali mirch) Fruit, [19] *Piper longum* (Pippali) Fruit [20, 21].
Pep-Up Syrup contains extract of *Withania somnifera* (Ashwagandha) Root, [22] *Plumbago zeylanica* (Chitrak) Root, [10, 11] *Apium graveolens* (Amjoda) Fruit, [23, 24] *Eclipta alba* (Bhringraj) Whole plant, [25] *Centella asiatica* (Madukparni) Whole plant, [26, 27] *Zingiber officinale* (Shunthi) Rhizome, [15, 16] *Piper longum* (Pippali) Fruit, [20, 21] *Punica granatum* (Dadim) Fruit bark, [28] *Alpinia galanga* (Kulinjan) Rhizome, [29] *Elettaria cardamomum* (Elaichi) Fruit, [30] *Mesua ferrea* (Nagkesar) Stamens **[31]** and *Cinnamomum zeylanicum* (Twak) Bark [32].
Majority of ingredients of Pep-Up Tablet and Syrup are well reported in Ayurvedic texts and scientific research publications for gastroprotective effect. However, no such evidence was found which proves efficacy of their combination. The present study was aimed to investigate

gastro-protective effect of Pep-Up Tablet and Syrup on water immersion plus restraint stress- induced gastric ulcer in rats.

2. Materials and methods

2.1 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) of 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.

2.2 Administration of test drug and dosage

The test drugs (Pep-Up Tablet and Syrup) were received from Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. Pep-Up Tablet was administered orally in form of suspension by mixing with vehicle solution of 1% sodium carboxy methyl cellulose (Na-CMC). Pep-Up Syrup was administered orally as it is. Dose of the test drugs were fixed by extrapolating the human dose to laboratory animals, based on body surface area ration as per the table of Paget and Barnes **[33]**. Experimental dose of Pep-Up Tablet was 200 mg/kg/day (p.o.) and Pep-Up Syrup was 3 mL/kg/day (p.o.).

2.3 Experimental groups

The selected animals were divided in to four groups where each group consisted of six animals.

Group 1: (Normal control): received distilled water as vehicle

Group 2: (Disease control): received distilled water as vehicle and exposed to water immersion plus restrain stress

Group 3: (Pep-Up Tablet treated): received Pep-Up Tablet (200 mg/kg/day, p.o.) and exposed to water immersion plus restrain stress

Group 4: (Pep-Up Syrup treated): received Pep-Up Syrup (3 mL/kg/day, p.o.) and exposed to water immersion plus restrain stress

2.4 Water immersion plus restraint stress-induced gastric ulcer

The selected animals were divided in to four groups as mentioned above. Pep-Up Tablet and Syrup were administered for 7 days through oral route in group 3 and 4 respectively. The method described by Takagi and Okabe [34] was employed with slight modification. After sixth day treatment rats were fasted for 18 h, care being taken to avoid coprophagy. The rats were immobilized in a restrainer and subsequently they were immersed in water up to xiphoid process for 7 h. The temperature of the water was maintained at 24±5 ^{0}C. Drugs were given orally 2 h prior to the restraint procedure. After 7 h of immobilization and water immersion the animals were taken out and killed with high-dose anesthetic ether. The stomach was removed and ulcer index with its severity **[35]** was determined. The samples of stomach tissue were analyzed to determine gastric wall mucus content, [36] reduced glutathione, [37] superoxide dismutase, [38] catalase activity [39] and lipid peroxidation (MDA) [40].

2.5 Statistical analysis

The data were expressed as mean ± standard error of mean (SEM) for six rats per experimental group. Different groups were compared with analysis of variance (ANOVA) followed by *post hoc* Tukey's test. A $P \leq 0.05$ was considered as statistically significant.

3. Results and discussion

The experimental stress ulcer may be considered equivalent to clinical stress ulcer. An acute gastric hemorrhagic lesion in the glandular stomach characterizes a stress ulcer [1]. In present study, exposure to water immersion plus restraint stress developed gastric ulceration and decreased content of gastric wall mucus. Pretreatment of Pep-Up Tablet and Syrup provided significant gastroprotection against water immersion plus restraint stress-induced gastric ulcer and mucosal damage. Pep-Up Syrup showed more significant protection against gastric ulcer and mucosal damage with respect to Pep-Up Tablet (Table 1).

Table 1: Effect of Pep-Up Tablet and Syrup on ulcer index and gastric wall mucus content

Group	Ulcer index	Gastric wall mucus content (µg/mL)
Normal control	0.00 ± 0.00	969.47 ± 09.14
Disease control	1.26 ± 0.11$^{###}$	631.23 ± 13.47$^{##}$
Pep-Up Tablet treated	0.78 ± 0.05**	858.08 ± 14.80**
Pep-Up Syrup treated	0.54 ± 0.05**	861.72 ± 11.73**

All the values are expressed as mean ± SEM (n=6) in each group. $^{**}P < 0.01$ when compared to disease control group; $^{##}P < 0.01$, $^{###}P < 0.001$ when compared to normal control group.

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. Exposure to water immersion plus restraint stress significantly reduced anti-oxidant parameters of stomach tissue viz. reduced glutathione, superoxide dismutase and catalase activity. Pre-treatment of Pep-Up Tablet and Syrup both showed significant protection against depletion of tissue anti-oxidants in comparison of disease control group however, Pep-Up Syrup was little superior as compared to Pep-Up Tablet (Table 2).

Table 2: Effect of Pep-Up Tablet and Syrup on anti-oxidant biochemical parameters of stomach tissue

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	893.82 ± 24.83	400.00 ± 28.58	910.99 ± 79.92
Disease control	496.76 ± 16.07$^{\#\#}$	176.96 ± 17.64$^{\#\#}$	407.74 ± 35.46$^{\#\#\#}$
Pep-Up Tablet treated	729.61 ± 18.81*	344.32 ± 17.64**	815.50 ± 67.38**
Pep-Up Syrup treated	814.41 ± 17.07**	358.24 ± 13.56**	891.60 ± 61.74***

All the values are expressed as mean ± SEM (n=6) in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.01$ when compared to disease control group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared to normal control group.

The observed significant anti-oxidant property of Pep-Up Tablet and Syrup may be attributed to *Withania somnifera* (Ashwagandha) Root, [22] *Zingiber officinale* (Shunthi) Rhizome, [15, 16] *Emblica officinalis* (Amalaki) Fruit, [5-7] *Terminalia chebula* (Haritaki) Fruit, [8, 9] *Piper longum* (Pippali) Fruit [20, 21] and *Piper nigrum* (Kali mirch) Fruit [19]. All these ingredients are well reported for having anti-oxidant property.

As a matter of fact, free radicals also has important role in the pathogenesis of various tissue injury, including the digestive system. Oxygen and hydrogen derived free radicals like hydrogen peroxide, hydroxyl radicals can be responsible for mucosal damage [41]. Pre-treatment of Pep-Up Tablet and Syrup both significantly inhibited lipid peroxidation in comparison to disease control group which indicates their certain effect against free radicals generation and related damage (Table 3) however Pep-Up Syrup was found more effective in comparison to Pep-Up Tablet.

Table 3: Effect of Pep-Up Tablet and Syrup on lipid peroxidation level of stomach tissue

Group	Lipid peroxidation (nmole/g of tissue)
Normal control	226.43 ± 13.77
Disease control	379.06 ± 19.26$^{\#\#}$
Pep-Up Tablet treated	308.23 ± 12.36*
Pep-Up Syrup treated	259.27 ± 24.72**

All the values are expressed as mean ± SEM (n=6) in each group. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to disease control group; $^{\#\#}P < 0.01$ when compared to normal control group.

4. Conclusion

From the present study, it can be concluded that Pep-Up Tablet and Syrup both are having significant anti-oxidant property which may be responsible for enhancing the stability of gastric mucosal tissues against damage caused by water immersion plus restraint induced stress. Promising gastro-protective effect of Pep-Up Tablet and Syrup on stress induced gastric ulceration was also found supporting to their acclaimed clinical application.

5. Acknowledgement

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

6. References

1. Brodie DA, Hooke KF. The effect of vasoactive agents on stress-induced gastric hemorrhage in the rat. Digestion 1971; 4:193-204.
2. Chul-Hong P, Hyung-US, Minsik S, Sang-Han L. Protective effect of Acer mono Max. sap on water immersion restraint stress-induced gastric ulceration. Exp Ther Med 2011; 2(5):843-848.
3. Estakhr J, Sanchooli N, NajafiSh, Javdan N. Anti-inflammatory activity of ethanolic extract of Physalisalkekengi. Res J Pharm Biol Chem Sci 2011; 2(3):421-425.
4. Schmeda-Hirschmann G, Yesilada E. Traditional medicine and gastroprotective crude drugs. J Ethnopharmacol 2005; 100:61-66.
5. Rao M, Siddiqui H. Pharmacological studies on Emblica officinalis Gaertn. Indian J Exp Biol 1964; 2:29-31.
6. Singh B, Sharma P. Effect of Amalaki on amalapitta. J Res Ind Med 1971; 5:223-229.
7. 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.
8. Suryaprakash DV, Sreesatya N, Avanigadda S, Vangalapati M. Pharmacological Review on Terminalia Chebula. Int J Res Pharm Biomed Sci 2012; 3(2):679-683.
9. Raju D, Ilango K, Chitra V, Ashish K. Evaluation of Anti-Ulcer Activity of Methanolic extract of Terminalia chebula Fruits in Experimental. J Pharm Sci Res 2009; 1(3):101-107.
10. 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.
11. Datta S, Mishra R. Plumbago zeylinica Linn. (Chitrak) - Review as Rasayan (Rejuvenator / Antiaging). Int J Res Pharm Biomed Sci 2012; 3(1):250-267.
12. Bairwa R, Sodha RS, Rajawat BS. Trachyspermum ammi. Pharmacogn Rev 2012; 6(11):56-60.
13. 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.
14. Pandey GS, Chunekar KC. Bhavprakash Nighantu of shri bhavamisra. Published by chaukhambha bharati academy, Varanasi, India, 2002, 165.
15. 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.
16. Chamundeeswari D, Kanimozhi P, Kumar V, Reddy C. Formulation and evaluation of Churna for digestive property. Sri Ramachandra J Med 2007; 39-43.
17. Pandey GS, Chunekar KC. Bhavprakash Nighantu of shri bhavamisra. Published by chaukhambha bharati academy, Varanasi, India, 2002, 154.
18. Pandey GS, Chunekar KC. Bhavprakash Nighantu of shri bhavamisra. Published by chaukhambha bharati academy, Varanasi, India, 2002, 161.
19. Boddupalli B, Ramani R, Subramaniam B, Anisetti R. In vitro and invivo evaluation of hepato protection and anti-ulcer activities of piperine gastro retentive micropspheres. Asian Pac J Trop Biomed 2012; S1237-S1240.
20. 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.
21. Agrawal AK, Rao CV, Sairam K, Joshi VK, Goel RK. Effect of Piper longum Linn, Zingiber officianalis Linn and Ferula species on gastric ulceration and secretion in rats. Indian J Exp Biol 2000; 38(10):994-998.
22. Bhatnagar M, Sisodia SS, Bhatnagar R. Antiulcer and antioxidant activity of Asparagus racemosus willd and withania somnifera dunal in rats. Ann N Y Acad Sci 2005; 1056:261-278.
23. Baananou S, Piras A, Morongiu B, Dessi MA, Falconieri D, Porcedda S *et al*. Antiulcerogenic activity of Apium graveolens seeds oils isolated by supercritical CO2. Afr J Pharm Pharmacol 2010; 6:756-762.
24. Fazal SS, Singla RK. Review on the pharmacognostical and pharmacological characterization of apium graveolens Linn. Indo Global J Pharm Sci 2012; 2:36-42.
25. Banerjee A, Shrivastava N, Kothari A, Padh H, Nivsarkar M. Antiulcer activity of methanol extract of Eclipta alba. Indian J Pharm Sci 2005; 67:165-168.
26. Abdulla MA, AL-Bayaty FH, Younis LT, Abu Hassan MI. Anti-ulcer activity of Centella asiatica leaf extract against ethanol-induced gastric mucosal injury in rats. J Med Plant Res 2010; 4:1253-1259.
27. Cheng CL, Koo MW. Effects of Centella asiatica on ethanol induced gastric mucosal lesions in rats. Life Sci 2000; 67:2647-2653.
28. Ajaikumar KB, Asheef M, Babu BH, Padikkala J. The inhibition of gastric mucosal injury by Punica granatum L. (pomegranate) methanolic extract. J Ethnopharmacol 2005; 96:171-176.
29. Al-Yahya MA, Rafatullah S, Mossa JS, Ageel AM, Al-Said MS, Tariq M. Gastric antisecretory, antiulcer and cytoprotective properties of ethanolic extract of Alpinia galanga willd in rats. Phyto Res 1990; 4:112-114.
30. Jamal A, Siddiqui A, Javed K, Jafri M. Antiulcerogenic activity of Elettaria cardamomum Maton. and Amomum subulatum Roxb. Seeds. Indian J Tra Knowl 2005; 4:298-302.
31. Gopalakrishnan C, Shankaranarayanan D, Nazimudeen S, Viswanathan S, Kameswaran L. Anti-inflammatory and CNS depressant activities of xanthones from Calophyllum inophyllum and Mesua ferrea. Indian J Pharmacol 1980; 12:181-191.
32. Tabak M, Armon R, Neeman I. Cinnamon extracts' inhibitory effect on Helicobacter pylori. J Ethnopharmacol 1999; 67:269-277.
33. Paget GE, Barnes JM. Evaluation of drug activities. In: Lawrence DR, Bacharach AL, editors. Pharmacometrics. Vol. 1, New York: Academic Press, 1964, 161.
34. Takagi A, Okabe S. The effects of drugs on the production and recovery processes of the stress ulcer. Jpn J Pharmacol 1968; 18:9-18.
35. 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.
36. Corne SJ, Morrissey SM, Wood RJ. A method for the quantitative estimation of gastric barrier mucus. J Physiol 1974; 242(2):116-117.
37. Beutler E. Red cell metabolism: A manual of Biochemical methods. Grune & Stratton, New York; 1971, 9-16.
38. Misra HP, Fridovich I. The role of Super oxide anion in the auto-oxidation of Epinephrine and a simple assay for Superoxide dismutase. J Biol Chem 1972; 247:3170-3175.
39. Samuel A, Bernard E. Photometric determination of catalyse activity. J Biol Chem 1950; 187:705-709.
40. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions *in vitro*. Biochem J 1971; 123:805-814.
41. Joseph RM, Varela V, Kanji VK, Subramony C, Mihas AA. Protective effects of zinc in indomethacin induced gastric mucosal injury: evidence for a dual mechanism involving lipid peroxidation and nitric oxide. Aliment Pharmacol Ther 1999; 13:203-208.