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Research Article

# Preparation and Evaluation of Nutraceutical Formulation for Ulcer Healing Activity

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**ABSTRACT**

Herbs, vegetables and fruits are being considered to be the potential source for treating and controlling various ailments. "Let food be thy medicine and medicine thy food" the tenet by Hippocrates is presently receiving great interest. In this contest the concept of functional food is highly appreciated. Functional foods are "food or food ingredient which provides both health and nutritional benefits." The purpose of the present study is to formulate and evaluate a formulation containing nutraceuticals for prevention and treatment of peptic ulcers. Factors like *H.pylori* infection, consumption of NSAID's, alcohol, tumors of the acid producing cells, smoking, chewing tobacco contributes for peptic ulcers. It is affecting millions of individuals worldwide. Conventional treatment includes the drugs which reduce the rate of gastric acid secretion, drugs which protects the mucus membrane that lines the GIT or the drugs which eliminates *Helicobacter pylori*. In most of these cases adverse reactions and incidence of relapse has been observed. The concern is to introduce safer drugs with maximum therapeutic effect, without adverse reactions. In the present study a formulation (PHF-1) containing selected functional foods which acts by various mechanisms to prevent and treat ulcers was developed and evaluated for its efficacy against ethanol induced gastric ulcers. Antiulcer potential was assessed by determining ulcer index, gastric pH, gastric juice volume and total acidity. The formulation was found to be on par with standard drug Sucralfate. The findings suggest that the formulation is worth as an alternative treatment for gastric ulcer.

**Keywords:** Nutraceuticals, Ethanol, PHF-1. Gastric ulcers, Antiulcer

## INTRODUCTION

Peptic ulcers are normally caused by the imbalance between the amount of acid that is produced in the stomach and the mucous defense barrier, resulting in the damage of mucosal lining of the stomach or duodenum[1]. The pathophysiology of peptic ulcer disease involves an imbalance between offensive factors like acid, pepsin and *H. pylori* and defensive factors like mucin, prostaglandin, bicarbonate, nitric oxide and growth factors[2]. Untreated ulcers can lead to serious health problems like intestinal bleeding, perforation in the intestinal lining, blood vomiting and gastric outlet obstruction. People of any age can be affected by stomach ulcers. An estimated 15,000 deaths occur each year as a consequence of peptic ulcer[3]. Conventional treatments include H2 blockers, proton pump inhibitors, antacid preparations and antibiotics to treat *H. pylori* infections. Possible side effects these drugs include constipation or diaarhoea, cramps, headache, joint pain or rebound acidity. Herbs being more acceptable by the body, with no or minimum toxicity and cultural acceptability have still preserved their potential as medicines. The growing acceptance of the role of diet in the prevention and treatment of diseases has motivated many manufacturers to venture into functional foods or nutraceutical formulations. Food therapy being the basic therapy in Chinese medicine uses selected food ingredients to prepare medicine for preventing or treating specific health conditions and these foods are now defined as nutraceuticals or functional foods. A functional food is a food given to promote good health or to prevent disease. As per new definition "Functional Food is a Natural or processed food that contains known biologically-active compounds which when in defined quantitative and qualitative amounts provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age". It was debated at the 9th International Conference on "Functional Foods and Chronic Diseases: Science and Practice" at the University of Nevada, Las Vegas on March 15-17, 2011[5]. Present study reports the potential antiulcer activity of formulation containing selected nutraceuticals.

## MATERIALS AND METHODS

*Formulation (PHF-1) development*

All the materials used in the formulation were purchased from the commercial market, Hyderabad.

*Preparation 1:* 20g each of coarsely powdered *Coriander sativum, Zingiber officinalis, Cuminum cyminum* were boiled in 100 ml of distilled water for 1 hour or until the water reduces to around 1/4th of its volume. The decoction is filtered and cooled to get a clear liquid.

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Table 1: Effect of PHF-1 on ulcer index, gastric pH, gastric volume and Total acidity in ethanol induced gastric ulcer model in rats.

| Group | Volume of gastric juice (ml) | pH | Total acidity mEq/L | Ulcer index | Percentage protection After 1hr. |
|---|---|---|---|---|---|
| Disease control | 2.1±0.02 | 2.02±0.02 | 150.42±8.02 | 11.32 | -- |
| Sucralfate | 0.8±0.22** | 5.92±0.32 | 76.02±2.42 | 5. 48±0.22 | 46.59** |
| PHF-1 (2ml/kg) | 1.4±0.32 | 3. 22±0,22 | 110.02±3.22 | 8.42±0.38 | 35.61 |
| PHF-1 (4ml/kg) | 1.0±0.24* | 5.42±0.02* | 80.62±3.18* | 5.82±0.42 | 48.58** |
| PHF-1 (8ml/kg) | 0.8±0.42** | 5.98±0.42** | 75.02±3.42** | 5.42±0.12** | 52.12** |

Values expressed as (Mean ± SEM), n=5, *p<0.05 **p<0.01 when compared with control group

*Preparation 2:* 25 gms of fully riped *Feronia elephantum* pulp was soaked for 24 hours in 100ml of warm distilled water and squeezed and filtered through muslin cloth.

*Preparation 3:* 10 gms of crushed seeds of *Trigonella foenum-graecum* is soked for 24 hrs in 50 ml of distilled water and filtered.

*Preparation 4:* 10ml of fresh juice of *Cucumis sativa* was prepared using commercial juicer. To this 10gms of *Musa sapientum* fine powder (prepared by drying fully ripe bananas in shade and powdered) and 100ml of commercial honey was added.

Finally, 25ml of preparation 1, 10ml of preparation 2, and 20ml of preparation 3 were mixed and added to preparation 4, mixed thoroughly using mixer for one minute. No preservative was added.

*Dose Used:* 2ml/kg bw, 4ml/kg bw and 8ml/kg bw. (Oral)

*Reference drug:* Sucralfate (5ml/kg bw)

*Control vehicle:* 0.9% NaCl. (2ml/kg bw)

*Evaluation of antiulcer activity*

Institutional animal ethical committee approval was taken to conduct the experiment at Bharat Institutions, Hyderabad.

*Ethanol induced ulcer model*

The method followed by Yara Cavalcante was adopted[6]. with modification. Wistar rats of either sex (200-250 g) housed under standard condition, in groups of five, with food and water *ad libitum* were used. The animals were kept on a 12 h light and 12 h dark regimen at 25°C prior to the experiments. The animals were deprived of food for 24 h before experiment but provided free access to water. Experimental animals were divided into six groups of five each. I, II and III groups received the formulation at the dose of 2ml/kg bw, 4ml/kg bw and 8ml/kg bw respectively. IV group received the standard drug sucralfate and V group received vehicle (ulcer control) 30 min before absolute ethanol (1ml /kg bw) treatment. VI group was taken as normal control received only vehicle. All treatments were made by gavage. After 1hr. of treatment the animals were sacrificed, stomach was removed by cervical dislocation and was excised along with greater curvature, contents were collected and the gastric lesions (number of ulcers) were counted using 10x magnifying lens. Scoring was done as follows:

- Normal coloured stomach---0
- Red colouration---0.5
- Spot ulcer----1
- Hemorrhagic streak---1.5
- Ulcers more than three and less than five---2
- Ulcers more than five-----3

Ulcer index was calculated using the formula $UI=Un+Us+Up \times 10^{-1}$

Where by

UI= Ulcer Index; UN = Average number of ulcers per animal; US =Average number of severity score; UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as follows:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index of Control} - \text{Ulcer index of Test}) \times 100}{\text{Ulcer index Control}}$$

*Estimation of gastric volume, pH and total acidity*

A method followed by Vinod Nair et.al[7] was adopted. The collected gastric content was transferred to graduated centrifuge tubes and centrifuged at 1000 rpm for 15min. Gastric volume was noted. The supernatant was collected and pH was determined using digital pH meter by diluting 1ml gastric juice with 1ml distilled water. Total acidity was determined by titrating 1.0ml of gastric juice against 0.01N NaOH using phenolphthalein indicator. Total acidity was calculated using the formula

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{Normality} \times 100 \text{ mEq/L}}{0.1}$$

*Statistical Analysis*

The results are expressed as the mean ± SEM for each group. Statistical differences were calculated using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Differences were considered to be statistically significant at $P \leq 0.05$.

**RESULTS**

Effect of PHF-1 on ulcer index, gastric volume, gastric pH and total acidity is shown in Table-1. Experimental results reveal that the ethanol treated group showed high ulcer index (11.32), low pH and caused more accumulation of gastric secretions compared to pre-treated drug and formulation groups. Ulcer index, total acidity and gastric volume was significantly reduced ($p<0.05$) and pH was increased in PHF-1 treated groups especially at the dose of

4ml/kg bw and 8ml/kg bw compared to untreated group. Activity was found to be dose dependent. Results were comparable with the standard drug sucralfate.

## DISCUSSION

In the present study, a formulation containing selected functional food reputed to be as antacids and antiulcer was developed and evaluated for antiulcer activity in ethanol induced ulcer model in rats. The ingredients selected were well known antiulcer drugs and carminatives, *viz Coriander sativum* [8], *Zingiber officinalis* [9], *Cuminum cyminum*[10], *Feronia elephantum* [11], *Trigonella foenum-graecum*[12], *Cucumis sativa*[13] *and Musa sapientum*[14]. Quantity was selected based on previous trials. These have traditionally been used by herbalists and indigenous healers for the prevention and treatment of peptic ulcers. The gastric lesions induced by ethanol is stasis in gastric flow leading to the development of haemorrhage and necrosis of tissue. Ethanol penetrates easily into the mucosal layer and causes intracellular permeability to sodium and calcium, excess of which results in mucosal injury and exfoliation. This reduces secretion of bicarbonates and mucus production and increases the neutrophil infiltration into gastric mucosa which adheres to endothelial cells, causing blockage of capillaries and induces damage to endothelial cells through the release of protease, leukotrienes and oxygen free radicals[15-17]. The ingredients used in the present formulation contain Honey which also acts as a vehicle. Honey is nutritive and acts as demulcent and provides soothing effect on the mucus membrane. *Musa sapientum* (banana) contains quercetin, leucocyanidin protects gastric mucosa from erosions[18]. Ginger appears to suppress inflammation due to the presence of phenolic compounds such as shogaols and gingerols[19,20]. Coriander contains different antioxidant constituents like linanool, flavonoids, coumarins, catechins, terpenes and polyphenolic compounds, which acts by scavenging free radicals produced and thus gives protective effect on gastric mucosa and the inhibition of ulcers might be due to the formation of a protective layer of either one or more than one of these compounds by hydrophobic interactions[21]. Gastro protective effect of cumin may be due to epoxy-carvone and other phenolic compounds may be by increasing the glutathione level (Ethanol decreases glutathione level)[22]. Cytoprotective effect of Cucumis may be due to the presence of flavonoids and polyphenolic acids, enzymes such as superoxide dismutase, glutamate transpeptidase, tannins which are powerful antioxidants. Cucumber is cooling and contains around 96% water and it is also rich in mineral content[13]. Cytoprotective effect of Trigonella (fenugreek) seeds may be due to its anti-secretory property and prevention of lipid peroxidation induced by ethanol, thus lowering the mucosal injury[23]. Fenugreek is a rich source of polysaccharide galactomannan. It also contains mucilage, such as choline and trigonelline which might have contributed for antisecretory activity. Protective effect of *Feronia elephantum* may be attributed to the presence of tannins. Fruit also contains fruit acids, vitamins and mineral. The dried pulp contains 15% of citric acid, potassium, calcium and iron salt. Seeds and fruits contained oil and protein; oil composed of palmitic, oleic, linoleic and linolenic acids[24].

## CONCLUSION

From the present study it can be concluded that the formulation developed could significantly protect gastric mucosal damage induced by ethanol. The protection was found to be dose dependent. Since the ingredients used acts by various mechanisms and also possess nutritional value, the present formulation can be considered as an alternative, cost effective, safer and potential medicine to treat gastric ulcers. Due to the increasing popularity with health-conscious consumers, functional food science is becoming popular and creating new interest in marketing the products. It is hoped that the study serve to develop cost effective, safer and potential formulation for peptic ulcer.

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