Research Article

The Effect Of Polyherbal Formulation - PHF On Experimentally Induced Reflux Esophagitis In Rats

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ABSTRACT

The science of life—*Ayurveda* is practiced in India since time immemorial. Besides being cheap and easily available, *Ayurvedic* drugs are considered as safe. Moreover, there is surge in the interest in *Ayurveda* due to quest of alternative medicines. In Ayurvedic system of medicine, Polyherbal formulations were frequently used to enhance the activity or counteract the toxic effect of compounds, from other plants, but may also act synergistically with other constituents from the same plants. Gastro esophageal reflux disease is a disorder of defense mechanism at the esophageal junction, caused by regurgitation of the gastric contents especially of gastric acid. The purpose of the study was to investigate the effect of Poly Herbal Formulation (PHF) on experimentally induced reflux esophagitis and gastrointestinal motility in animals. The PHF consists of seven medicinal plants namely *Aegle marmelos*, *Elettaria cardamomum*, *Glycyrrhiza glabra* , *Citrus aurantifolia* , *Rosa damascena*, *Cissus quadrangularis* and *Saccharum officinarum*. Based on acute toxicity study the PHF was considered as safe and 3 dose (100, 200 and 400 mg/kg) levels were employed for pharmacological evaluation. The test drugs were administered orally by suspending in 1% carboxy methyl cellulose solution. The PHF exhibited ($P<0.001$) significant decrease in lesion index and enhance the % protection of lesion in experimentally induced reflux esophagitis at all the 3 doses in rats. In charcoal meal gastrointestinal transit test, PHF dose-dependently propelled the charcoal meal travel through the small intestines in mice. The study indicates that the PHF has protective effect against surgically induced reflux esophagitis.

Keywords: Polyherbal formulation, esophagitis, gastrointestinal motility and Charcoal meal

INTRODUCTION

Gastro esophageal reflux disease (GERD) is a disorder of defense mechanism at the esophageal junction, caused by regurgitation of the gastric contents, especially of gastric acid. GERD is associated with decreased gastric emptying and/or increased incidence of transient lower esophageal relaxation[1]. If left untreated, erosion and ulceration of the esophageal mucosa by gastric acid, chronic esophagitis, aspiration pneumonia, esophageal strictures and Barrett's esophagus (a premalignant condition) may result [2]. Besides that, disturbed gastrointestinal motility and injurious effects of the acid-peptic refluxate on the esophageal epithelium are also responsible for GERD symptoms. Hence along with prokinetic drugs, suppression of gastric acid is the current pharmacotherapeutic approach for its treatment [3].

There are many drugs used for the treatment of acid peptic disorder, such as antacids, proton pump inhibitors or antihistaminic agents and gastroprokinetic agent such as mosapride or metoclopramide, but most of these drugs produce several adverse reactions. Thus, there is a need of more effective and less toxic agents for this ailment. Plants are some of the most attractive sources, and have been shown to produce promising results for the treatment of various GIT disorders. Even though single herbal is effective in the treatment of human ailments, but drugs with multiple mechanisms of protective action may be one way forward in minimizing tissue injury in human disease [4].

PHF used in this study consists of seven medicinal plants namely *Aegle marmelos, Elettaria cardamomum, Glycyrrhiza glabra, Citrus aurantifolia, Rosa damascena, Cissus quadrangularis* and *Saccharum officinarum.* The indigenous plants used in the formulation have been recognized to treat various gastrointestinal illnesses. Warrier et al mentioned the uses of plants listed in PHF in various gastrointestinal ailments, which was given in Table I. Based on previous reports, the present study was aimed to investigate the effect of PHF on experimentally induced reflux oesopagitis as well as gastrointestinal motility in rats[5].

MATERIALS AND METHODS

Plant material

The plants *Aegle marmelos, Elettaria cardamomum, Citrus aurantifolia, Rosa damascena, Cissus quadrangularis* and *Saccharum officinarum* were collected locally during the month of December and *Glycyrrhiza glabra,* obtained from the Horticulture Research Station, Kodaikanal, Tamilnadu. Prof. R. Duraisamy, Botanist., authenticated the botanical identity of the plants and voucher specimen (NCP/Phcog/2008/ 0201) has been retained, for future reference in the herbarium of Pharmacognosy department, Nandha College of Pharmacy, Erode, India.

Formulation development

The collected plant materials were shade dried separately and pulverized to get a fine powder. The fine powders were passed through the sieve number 100. The graded powders were weighed individually and mixed to give the specified composition. This herbal formulation was stored in air tight container and used for pharmacological studies. The PHF was administered orally to the animals by suspending in 1 % carboxy methyl cellulose solution.

Composition

Each gram of PHF contains powders of *Aegle marmelos* Corr. (Rutaceae; fruit, 150 mg), *Elettaria cardamomum* Maton. (Zingeberaceae; seeds, 125 mg), *Glycyrrhiza glabra* L. (Papilionaceae; root, 150 mg), *Citrus aurantifolia* Swingle. (Rutaceae; Fruits, 150 mg), *Rosa damascena* Mill. (Rosaceae; flower petals, 150 mg), *Cissus quadrangularis* Linn. (Vitaceae; Whole Plant, 150 mg) and *Saccharum officinarum* Linn (Poaceae; root, 125 mg).

Animals

Male Swiss albino mice weighing between 20 – 25 gm and male Wistar rats weighing between 150 – 220 gm were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2oC and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/2/C-CPCSEA) and were in accordance with the Institutional animal ethical guidelines.

Acute toxicity study

Acute toxicity studies were performed according to OECD-423 guidelines [6]. Male Swiss albino mice, selected by random sampling technique, were employed in this study. The animals were fasted for 4h with free access to water only. PHF was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 2000 mg/kg) doses of PHF were employed for further toxicity studies. During the study, the animals were closely observed for the first 12 hours for any toxic symptoms and 72 hours for mortality rate.

Evaluation of reflux esophagitis

The animals were divided into five groups each consisting of six rats. Group 1, represented control group of animals, received suspension of 1% carboxy methyl cellulose (CMC) in distilled water. Group 2 received Omeprazole (10 mg/kg). Groups 3–5 received PHF in doses of 100, 200and 400 mg/kg. The drugs were administered for three days, orally by suspending in 1% CMC solution. On day 3, after the last dose, the rats were kept for 12 hour fasting and care was taken to avoid coprophagy. Under ether anesthesia, the abdomen

was incised along the midline and both the pylorus and limiting ridge (transitional region between the forestomach and corpus) were then ligated simultaneously [7]. An approximately 1 cm long longitudinal cardiomyotomy across the gastroesophageal junction was performed to enhance reflux. Six hours later, the rats were sacrificed by a cervical dislocation, and the esophagus was harvested. The total area of the lesion that had developed in the esophagus was determined using a magnifying glass and the diameter of the lesion was measured using a vernier caliper. Lesion index was determined by following the scoring method of Suzuki et al [8].

Score 1: maximal diameter of 1 mm.
Score 2: maximal diameter of 1–2 mm.
Score 3: maximal diameter of 2–3 mm.
Score 4: maximal diameter of 3–4 mm.
Score 5: maximal diameter of 4–5 mm.
Score 10: an ulcer over 5mm in diameter.
Score 25: a perforated lesion.

Charcoal meal gastrointestinal transit test

Charcoal meal test was used to evaluate the gastrointestinal motility of PHF [9]. Mice were divided into five groups of six mice each and fasted for 24 h before the experiment. Group 1 served as control with suspension of 1% CMC in distilled water (10 ml/kg), group 2 was administered carbachol (1 mg/kg), a standard cholinergic agent, as the positive control. Groups 3 - 5 were then treated orally with three increasing doses of the PHF 100, 200and 400 mg/kg. The drugs were administered by suspending in 1% Carboxy methyl cellulose solution. After 15 min, the animals were given with 0.3 ml of freshly prepared charcoal meal (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal and 20% starch). Following 30 min of charcoal administration, the mice were sacrificed by cervical dislocation and the abdomens immediately opened to excise the whole small intestine (pylorus region to cecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured for obtaining the charcoal transport ratio or percentage.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. *P* values <0.05 were considered significant.

RESULT

All the doses (5, 50, 300, 2000 mg/kg) of PHF employed for acute oral toxicity studies were found to be non-toxic. PHF did not produce any mortality even at the highest dose (2000 mg/kg) employed. Three sub maximal doses (100, 200 and 400 mg/kg) which was found to be safe were employed for further pharmacological investigations.

Reflux esophagitis

The effect of PHF on surgically induced

Table-I. Shows the Ethnobotanicol uses of plants present in Polyherbal formulation

Plant name and Family	Ethnobotanicol Uses
***Aegle marmelos* Corr**. Rutaceae	Dyspepsia, Stomachalgia, Gastric irritability, Digestive, Laxative
***Elettaria cardamomum* Maton**. Zingeberaceae	Carminative, Digestive, Stomachic, Dyspepsia, Gastropathy, Hyperdipsia
***Glycyrrhiza glabra* L**. Papilionaceae	Laxative, Hyperdipsia, Gastralgia, Gastric ulcers
***Citrus aurantifolia* Swingle**. Rutaceae	Laxative, Appetiser, Stomachic, Digestive, Anthelmintic, Dyspepsia, Flatulance, Helmenthiasis,
***Saccharum officinarum* Linn**. Poaceae	Laxative, Dipsia, Gastropathy,
***Cissus Quadrangularis* Linn**. Vitaceae	Laxative, Anthelmintic, Carminative, Digestive, Stomachic, Helminthiasis, Anorexia, Dyspepsia, Flatulance, Chronic ulcers, Haemorrhoids,
***Rosa damascena* Mill**. Rosaceae	Laxative

Table-II: Protective effect of poly herbal formulation on experimentally induced Reflux esophagitis in rats

Drug treatment	Ulcer Index	% Protection
Control (1% Carboxy methyl cellulose)	8.50 ± 0.43	—
Omeprazole (10 mg /kg)	1.33 ± 0.21***	84.35
PHF 100	5.67 ± 0.56***	33.29
PHF 200	1.83 ± 0.31***	78.47
PHF 400	1.50 ± 0.22***	82.35

Values are presented as mean ± SEM (n = 6)

***P<0.001 Vs control; **P<0.01 Vs control; *P<0.05 Vs control

esophagitis was studied in rats. The effect of PHF on lesion index and % protection against surgically induced esophagitis is shown in table II. The PHF showed dose dependent decrease in lesion index. The PHF showed significant protective effect at the doses of 100, 200 and 400 mg/kg body weight. The percentage protection of lesion was 33.29 % (P< 0.01), 78.47% and 82.35% (P< 0.001) by PHF at the doses of 100, 200 and 400 mg/kg respectively; the percentage protection of lesion by Omeprazole, the standard antiulcer agent was 84.35% (P< 0.001) at the dose of 10mg/kg.

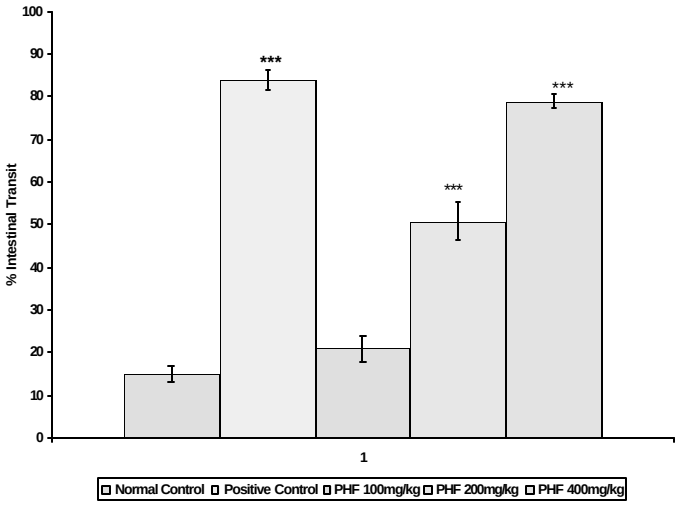

Figure I. Shows the effect of PHF the charcoal meal transit in mice.

***P<0.001 Vs control; **P<0.01 Vs control; *P<0.05 Vs control

Charcoal meal gastrointestinal transit test

Figure I. Shows the activity of the PHF to influence gastrointestinal transit of the charcoal meal in mice. It was determined by its effect on the traverse of charcoal meal through the length of the small gut of mice. PHF dose-dependently propelled the charcoal meal travel through the small intestines. The distance traveled by the solvent control was 14.93±1.89 %. The PHF at the dose of 100 mg/kg moved the charcoal meal to 20.97±2.99 %, while 50.76±4.56% and 78.95±1.66% (P< 0.001) was caused with the next higher doses (200 mg/kg and 400 mg/kg respectively). Carbachol (1 mg/kg) was used as positive control which moved the meal to 83.85±2.66% (P< 0.001).

DISCUSSION

Gastro-esophageal reflux disease is a common condition with a complex pathophysiology. Despite the wide range of abnormalities, since gastric acid plays a key role in mucosal damage, the suppression of gastric acid secretion is the basis of many treatments [10]. Additionally, promotility agents increase lower esophageal sphincter pressure and enhance lower esophageal peristalsis, both actions are specifically target the pathology of GERD. PHF, a Polyherbal formulation, consist of plants that are mentioned in Indian system of medicine (Ayurveda) for their curative properties. The anti-ulcer activity of PHF has been proved against

different experimental gastric and duodenal ulcers [11]. It appears to strengthen the gastric mucosal resistance and scavenging the free radical generation. The results showed that the protective effect of PHF against surgically induced reflux esophagitis in rats may be due to the inhibition of gastric secretion and the prevention of oxidative stress.

Gastrointestinal prokinetics promote the coordination of the gut wall contractions leading to enhancement of propulsive motility and consequently caudal displacement of luminal contents. Currently, they are considered as drugs of choice for the treatment of upper gastrointestinal tract functional and motor disorders such as those associated with gastro oesophageal reflux disease, chronic dyspepsia and acute or chronic idiopathic intestinal pseudo obstruction [12]. Charcoal meal gastrointestinal transit test was adopted for prokinetics activity of PHF in rats. Gastrointestinal transit was enhanced by PHF at all the three doses employed. The enhanced gastrointestinal transit by the PHF may be mediated through increased of prostaglandin synthesis. Prostaglandin was shown to contract the intestinal longitudinal muscle and relax the circular muscle *in vitro*, acting seemingly at pre and postganglionic sites in the intramural plexus [13]. The increase production of prostaglandin by PHF was already described by its anti-ulcer activity against indomethacin induced ulcer in rats [11].

The study indicates that the PHF has protective effect against surgically induced reflux esophagitis. It may be due to its gastro protective, anti-oxidant and prokinetics activity. However, further study is necessary to pinpoint the mechanism of reflux esophagitis of PHF and to identify the active constituents responsible for its activity.

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