

PHARMACOLOGICAL STUDY ON PHF- A NOVEL PROKINETIC POLYHERBAL FORMULATION

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Summary

The science of life—*Ayurveda* is practiced in India since time immemorial. Besides being cheap and easily available *Ayurvedic* drugs are considered 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. Polyherbal formulations (PHF) consist of seven known herbs namely, *Aegle marmelos*, *Elettaria cardamomum*, *Glycyrrhiza glabra*, *Citrus aurantifolia*, *Rosa damascena*, *Cissus quadrangularis* and *Saccharum officinarum*. The gastrointestinal prokinetics effect of PHF in various dose levels (100mg, 200mg and 400 mg/kg) was studied by charcoal meal gastrointestinal transit and laxative effect in mice. Carbachol (1 mg/kg) and Sennoside B (50 mg/kg) were used as reference drug. The test drugs were administered orally by suspending in 1% carboxy methyl cellulose solution. The results illustrate, PHF at 200mg and 400 mg/kg significantly ($p < 0.001$) enhanced the gastrointestinal transit. PHF at 400 mg/kg ($p < 0.01$) significantly enhanced the purging index which is the measure of laxative activity. PHF at 200 mg/kg ($p < 0.05$) less significantly enhanced the purging index. From the above findings we conclude that PHF may be used as gastrointestinal prokinetics.

Key words: PHF, Polyherbal formulation, prokinetics and gastrointestinal motility

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Introduction

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 drugs of choice for the treatment of upper gastrointestinal tract functional, motor disorders such as those associated with gastro oesophageal reflux disease, chronic dyspepsia, and acute or chronic idiopathic intestinal pseudo obstruction (1).

Although several chemicals and drugs are generally used against intestinal dysmotility and related intestinal diseases, the Indigenous drugs with a long descended heritage of traditional use are of supreme importance, to re-establish traditional claims with scientific interest. Herbal medicines are obtained from various plants and contain complex extracts with a large number of different active substances. The combination of herbs with various gastrointestinal active ingredients appears to be advantageous for a heterogeneous condition such as functional dyspepsia, gastro oesophageal reflux disease and intestinal related disorders. In the present study, the prokinetics effect of polyherbal formulation (PHF) on gastrointestinal motor function was investigated. The herbal formulation 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 ethnomedical uses and biological activity of the medicinal plants present in the PHF is given in the table 1. The prokinetics activity of PHF was evaluated *in-vivo* by intestinal transit rate of charcoal meal and laxative activity in mice.

Table: 1. Ethnobotanical / Ethnopharmacological uses/ Biological activity of plants present in Polyherbal formulation

Plant name and Family	Parts used	Ethnobotanical / Ethnopharmacological uses/ Biological activity	References
<i>Aegle marmelos</i> Corr. Rutaceae	Roots, Leaves and fruits	Dyspepsia, Stomachalgia, Gastric irritability, Digestive, Laxative Hypoglycaemic Anti ulcer	(2) (3) (4)
<i>Elettaria cardamomum</i> Maton. Zingiberaceae	Seeds	Carminative, Digestive, Stomachic, Dyspepsia, Gastropathy, Hyperdipsia Gastro protective activity	(2) (5)
<i>Glycyrrhiza glabra</i> L. Papilionaceae	Roots	Emetic, Diuretic, Laxative, Aphrodisiac, Hyperdipsia, Cough, Bronchitis, Gastralgia, Gastric ulcers	(2)
<i>Citrus aurantifolia</i> Swingle. Rutaceae	Fruits	Laxative, Appetiser, Stomachic, Digestive, Anthelmintic, cough, Bronchitis, Dyspepsia, Flatulance, Helmenthiasis,	(2)

<i>Rosa damascena</i> Mill. Rosaceae	Flowers	Abdominal and Chest pain,	(6)
		Strengthening the heart	
		Menstrual bleeding and Digestive problem	(7)
		Anti inflammatory activity	(8)
		Cough remedy	(9)
		Laxative	(10)
<i>Saccharum officinarum</i> Linn. Poaceae	Roots & Stems	Diuretic, Laxative, Cardiotonic,	(2)
		Aphrodisiac, Expectorant,	
		Haemostatic, Dipsia, Gastropathy, Erysipelas	
		Cholesterol Lowering effect,	(13, 14, 15)
		Inhibition of Platelet aggregation,	
		Antioxidant activity	
<i>Cissus Quadrangularis</i> Linn. Vitaceae	Whole Plant	Laxative, Anthelmintic,	(2)
		Carminative, Digestive, Stomachic,	
		Aphrodisiac, Helminthiasis,	
		Anorexia, Dyspepsia, Flatulance,	
		Chronic ulcers, Tumours,	
		Haemorrhoids, fractures etc. Gastro protective activity	(16)

Materials and Methods

Plant material

Each gram of polyherbal formulation (PHF) contains powders of *Aegle marmelos* Corr. (Rutaceae; fruit, 150 mg), *Elettaria cardamomum* Maton. (Zingiberaceae; 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). The plant materials were procured from a local supplier. 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.

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±2°C 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 ethical guidelines.

Acute toxicity study

Acute toxicity studies were performed according to OECD-423 guidelines (17). Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4h with free access to water. PHF was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was 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.

Charcoal meal gastrointestinal transit test (18)

Mice (20–30 g) were divided into five groups of six mice each and fasted for 24 h before the experiment. Group I served as control with suspension of 1% CMC in distilled water (10 ml/kg, p.o.), group II was administered carbachol (1 mg/kg, p.o.), a standard cholinergic agent, as the positive control. Groups III - V were then treated orally with three increasing doses of the PHF, 100, 200 and 400 mg/kg. The drugs were administered by suspending in 1% Carboxy methyl cellulose solution. After 15 min, the animals were given 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.

Screening for laxative activity (19)

Mice were fasted for 18 h and placed in individual observation cages, which were lined at the bottom with pre-weighed sheets of white absorbent paper. Six animals in individual cages were considered as a group and a total of 36 animals were thus divided into 5 groups. The first group served as the control. The second group received sennoside B (50 mg/kg, p.o.), the standard drug for comparison of potencies. Groups 3–5, received PHF, in doses of 100, 200 and 400 mg/kg. The drugs were administered orally by suspending in 1% Carboxy methyl cellulose solution. The animals were examined for hourly laxation for 5 h with the withdrawal of food and water. The percentage of respondents [*i.e.* (number of mice exhibiting laxation /total number of mice tested) X 100], latent periods (min) and number of wet feces passed during the test period were

noted for each group and from the data; purging index (PI) was calculated. Laxation was evidenced by any increment of purging indices as compared to the control.

Statistical analysis

The values were expressed as mean \pm 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.

Results

Effect of PHF on acute toxicity study

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 were found to be safe were employed for further pharmacological investigations.

Effect of PHF on charcoal meal gastrointestinal transit test

Figure. 1. Shows the activity of the PHF to influence the charcoal meal gastrointestinal transit 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 $50.76 \pm 4.56\%$ and $78.95 \pm 1.66\%$ ($P < 0.001$) was caused with the next higher doses (400 mg/kg and 200 mg/kg respectively). Carbachol (1 mg/kg) was used as positive control which moved the meal to $83.85 \pm 2.66\%$ ($P < 0.001$).

Effect of PHF on screening for laxative activity

The animals were examined for hourly laxation for 5 h after the administration of test drugs and expressed as purging index was given in figure 2. From the result of laxative screening, the PHF was found to exhibit laxative activity 400 mg/kg, from 120 minutes onwards and initially there was no laxation and data arisen from 120 to 300 min were statistically significant ($p < 0.01$). The PHF at lower dose levels of 200 mg/kg, also exhibited laxation but the results are less significant ($p < 0.05$) when compare to control. The PHF at still lower dose level of 100 mg/kg, did not exhibited significant laxation. The PHF increased the wetness of faecal droppings at 400 mg/kg. The reference standard sennoside B exhibited significant ($p < 0.001$) laxative property

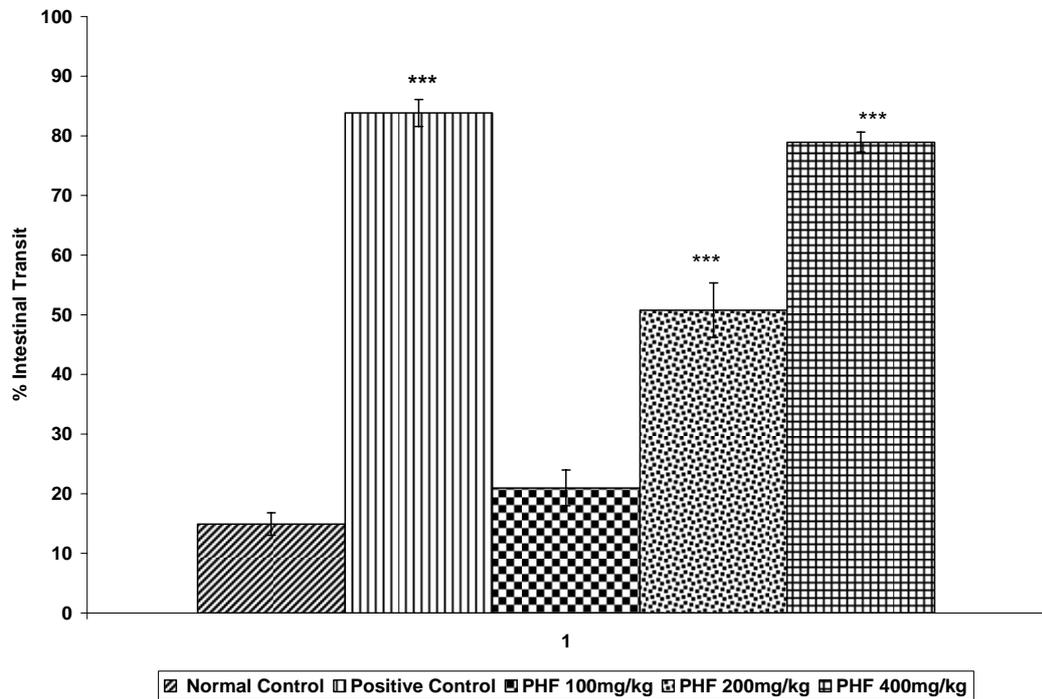


Figure.1 Shows the effect of PHF the charcoal meal transit in mice ^{***}P<0.001 Vs control; ^{*}P<0.01 Vs control; ^{*}P<0.05 Vs control

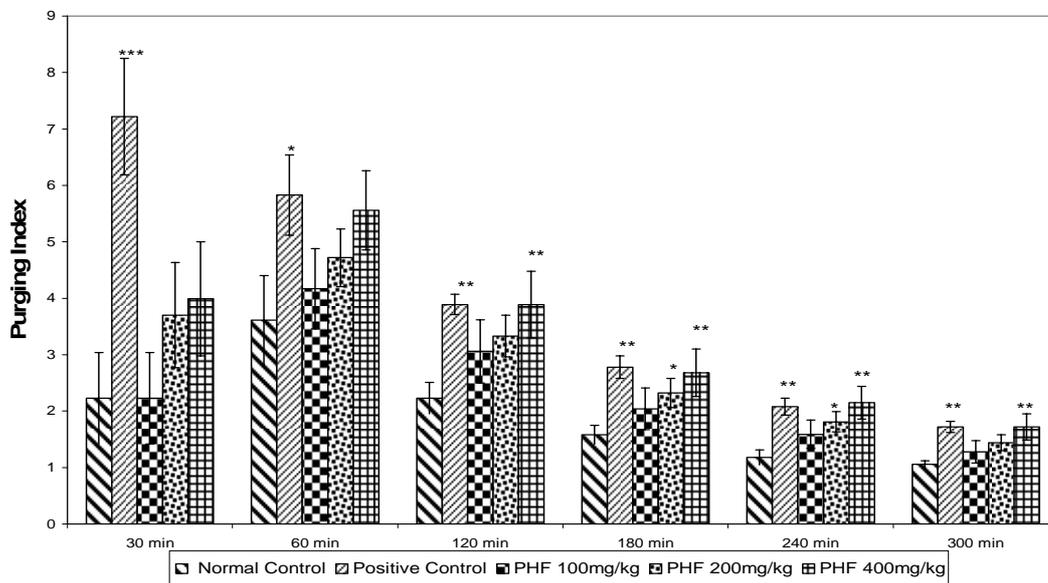


Figure.2 Shows the effect of PHF on the purging index in rats. ^{***}P<0.001 Vs control; ^{**}P<0.01 Vs control; ^{*}P<0.05 Vs control

Discussion

Propulsive motility is termed peristalsis and is sub served by a complex pattern of neural reflexes that aim to relax intestinal muscle downstream (descending inhibitory reflex) and contract the muscle upstream (ascending excitatory reflex) of the intestinal bolus. Intestinal transit is controlled by both neural and myogenic mechanisms (20). An increase of the contractile activity of the smooth muscle layers is in general responsible for acceleration of intestinal propulsion. Several mediators and neurotransmitters govern these motor patterns. Acetylcholine is the main excitatory neurotransmitter in the enteric nervous system, whereas NO is the major transmitter of the inhibitory motor neurons (21).

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 and relax the circular muscle *in vitro*, acting seemingly at pre and postganglionic sites in the intramural plexus (22). The increase production of prostaglandin by PHF was already described by its anti-ulcer activity against indomethacin induced ulcer in rats (23).

Purging index is the measure of the extent of laxation. Increased in purging index is observed with increased in the transit of gastrointestinal tract. Carbachol, a cholinergic agent, enhance the purging index and wetness of faces which is a reliable marker for increased gastrointestinal transit and secretion. Higher doses of PHF augment the frequency of defecation and propulsion of gastrointestinal tract.

From the above we conclude that PHF showed no acute oral toxicity in mice for a dose of 5 gm/kg. PHF accelerated the intestinal transit; moreover the references support the antiulcer property of the herbs present in the polyherbal formulation. These observations suggest that PHF can be used as prokinetics and in the treatment of gastrointestinal motility disorders like gastro oesophageal reflux disease, chronic dyspepsia etc.

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