PROTECTIVE EFFECT OF POLYHERBAL FORMULATION ON EXPERIMENTALLY INDUCED ULCER IN RATS

Srinivasan. D[1], Ramasamy. S[2], Sengottuvelu. S[#]

[1]Department of Pharmacology, Rajah Muthiah Dental College & Hospital, Annamalai University, Annamalainagar-608 002, Tamilnadu, India.

[2]Department of Pharmacology, Arupadai Veedu Medical College & Hospital, Pondicherry- 607 402, India

[#]Department of Pharmacology, Nandha College of Pharmacy, Erode-638 052, Tamilnadu, India.

Summary

In the 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. Therefore, the purpose of the study was to formulate a polyherbal formulation and investigate its anti-ulcer effect on various ulcer models such as pylorus ligation, indomethacin, cold restraint stress, HCl/Ethanol and cysteamine induced ulcers in animals. The Polyherbal formulation (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 used. Oral administration of PHF, once daily for 3 days showed dose-dependent anti-ulcer activity. The PHF exhibited significant (P<0.001) decrease in ulcer index and enhance the % protection of ulcer at all the 3 doses. Further study on gastric mucosal factors showed that it significantly decreased the offensive factors like acid (control: 214.08±5.65, PHF: 109.65±4.42) and pepsin (control: 49.35±1.35 and PHF: 20.00±0.92). PHF increased the defensive factors like mucin secretion (TC:P ratio of control: 1.55±0.06 and PHF: 4.24±0.07) and life span of mucosal cells (DNA content of gastric juice in control: 228.80±3.12 and PHF: 119.82±6.57). PHF showed significant antioxidant effect in stressed animals (Stress control: LPO, SOD and CAT were 242.92 ±7.99, 149.70±3.10 and 20.40±1.58 respectively; PHF reduced the LPO and SOD to 157.92±7.26 and 117.88 ±4.42 respectively and increase in mucosal CAT to 27.18±1.91). PHF did not have any effect on cell proliferation in terms of DNA mg/mg protein or glandular weight. The hexosamine (Stress control 20.48±1.22: PHF 100, 200and 400mg/kg were 28.17±0.71, 32.35±1.18 and 36.13±0.43 respectively) and gastric wall mucus (Stress control 812.47±41.54: PHF 100, 200and 400mg/kg were 997.82±14.32, 1042.5±20.73 and 1196.8±13.17 respectively) was significantly (P<0.001) increased by the PHF. From the results, it was concluded that PHF had significant anti-ulcer effects and this might be due to its effects on both offensive and defensive mucosal factors as well as scavenging of free radicals generation.

Key words: Polyherbal formulation, Anti-ulcer, *Aegle marmelos*, *Elettaria cardamomum*, *Glycyrrhiza glabra* , *Citrus aurantifolia*, *Rosa damascena*, *Cissus quadrangularis* and *Saccharum officinarum.*

Introduction

Gastric ulcers, one of the most widespread disease states, are believed to be due to an imbalance between acid and pepsin along with weakness of the mucosal barrier. There are many products used for the treatment of gastric ulcers, such as antacids, proton pump inhibitors or antihistaminic agents, but most of these drugs produce several adverse reactions. Thus, there is a need for more effective and less toxic anti-ulcer agents. Plants are some of the most attractive sources, and have been shown to produce promising results for the treatment of gastric ulcer. 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. It has been demonstrated that many drugs or formulations possess potent anti-oxidant actions and are effective in healing experimentally induced gastric ulcers. The herbal formulation derived from ayurveda, the traditional system of Indian medicine, has been found to have antiulcer properties. 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 indigenous plants used in the formulation have been recognized to treat various gastrointestinal illnesses. The anti-ulcer activity of Aegle marmelos (1), Glycyrrhiza glabra (2, 3), Elettaria cardamomum (4) and Cissus quadrangularis (5,6) have been reported. The seed powder of *Elettaria cardamomum* is frequently prescribed in the treatment of gastrointestinal disorder and is used as stomachic, resolvent, retentive, digestive, anti-emetic and carminative (4). It has also been mentioned that *Elettaria cardamomum* is used in the treatment of acid peptic disorders and gastritis (7). The citrus species have been reported to possess *in vitro* anti-*Helicobacter pylori* activity (8). There is a growing interest in citrus fruits because consumption of them appears to be associated with lower risk of colorectal (9), esophageal (10, 11) gastric (12), and stomach cancers (13). *Rosa damascena* is used as a gentle laxative (14). Warrier et al., (15) mentioned the uses of plants listed in polyherbal formulation in various gastrointestinal ailments, which was given in Table 1.

Based on previous reports, the experimental study was designed to evaluate the mechanism of anti-ulcer activity of the polyherbal formulation. The study includes the evaluation of anti-ulcer effect of PHF in pyloric ligation, indomethacin induced ulcer, cold restraint stress ulcer, HCl/EtOH-induced gastric ulcers and cysteamine induced duodenal ulcers. Pyloric ligation was adopted to study the anti-secretary property. Using HCl/EtOH, the antioxidant activity was analyzed. Indomethacin and stress ulcer models included to evaluate the cyto-protective effect. Cysteamine was used to evaluate the duodenal ulcers. This study provides an approach on the mechanism of the anti-ulcer effect of PHF.

Materials and Methods

Plant material

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.

Table-1. 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, Appetizer, Stomachic, Digestive, Anthelmintic, Dyspepsia, Flatulence, Helmenthiasis,
***Saccharum officinarum* Linn**. Poaceae	Laxative, Dipsia, Gastropathy,
Cissus Quadrangularis Linn. Vitaceae	Laxative, Anthelmintic, Carminative, Digestive, Stomachic, Helminthiasis, Anorexia, Dyspepsia, Flatulence, Chronic ulcers, Hemorrhoids,

* *Rosa damescena* not mentioned

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 herbal formulation was administered orally to the animals by suspending in 1 % carboxy methyl cellulose solution.

Composition

Each gram of polyherbal formulation (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±2^{o}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 (16). 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 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.

Pyloric Ligation Induced Ulcer

The method of Shay rat ulcer was adopted (17). 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 18 h fasting and care was taken to avoid coprophagy. The animals were anesthetized with anesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. 4 h after pylorus ligation the rats were sacrificed and the stomachs were dissected out and contents were collected in tubes for estimation of biochemical parameters. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers was measured using a vernier caliper. Ulcer index was determined by following the scoring method of Suzuki et al., (18).

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 ulcer.

Gastric Secretion Study

The gastric juice was collected 4 h after pyloric ligation and centrifuged for 5 min at 2000 rpm. The volume of the supernatant is expressed as ml/100 g body weight while free acidity and total acidity were determined by titrating with 0.01 M NaOH using Toepfer's reagent and phenolphthalein as indicator (19) and expressed as µEq/4 h. Peptic activity was estimated following the method of Debnath et al (20) and expressed as µg/ml. Dissolved mucosubstances like protein (21), total hexoses (22), hexosamine (23), sialic acid (24) and fucose (25), were estimated and the results are expressed in µg/ml. The ratio of total carbohydrates (TC, sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity (26). DNA content was estimated and expressed as µg/ml gastric juice 100 g weight of rats (27).

Cell Proliferation

Estimation of DNA in Gastric Mucosa: DNA (28) and protein (21) were estimated in the gastric fundal mucosal scrap homogenized in 2.5 ml of ice cooled 0.6N perchloric acid (PCA). The concentration of DNA is expressed as µg DNA/mg protein.

Measurement of Glandular Weights of Stomach: The weight of the glandular portion of stomach was calculated by subtracting the weight of the whole stomach minus rumen and is expressed as mg/100 g body weight of animals.

Indomethacin Induced Ulcer

The animals were divided into five groups each consisting of six rats. Group 1 represented control group of animals received suspension of 1% CMC in distilled water Group 2 received Omeprazole (10mg/kg). Groups 3–5, received PHF, in doses of 100, 200and 400 mg/kg. The drugs were administered for 3 days, orally by suspending in 1% CMC solution. On day 3, indomethacin (30 mg/kg) was given intra-peritonealy as a single dose to induce gastric ulcers (29) after 30 minutes of drug treatment. After 5 h, the animals were killed and ulcer index were scored as described earlier (18).

Cold Restraint Stress Induced Ulcers

The animals were divided into six groups each consisting of six rats. Group 1 represented normal control (non-ulcerated) animals received suspension of 1% CMC in distilled water. Group 2 represented as stress control animals received suspension of 1% CMC in distilled water. Group 3 received Omeprazole (10 mg/kg). Groups 4–6, 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, for 18 h fasted rats, cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2 h at 4–6 °C. The animals were then sacrificed by cervical dislocation. The stomach was taken out and cut open along the greater curvature and observed for ulcers. Ulcer index was scored as described earlier (18).

Estimation of free radical generations:

The fundus of stomach was used of estimation of free radical generation. The fundic part of the stomach was homogenized (5%) in ice cold 0.9% saline with a glass homogenizer. The homogenate was then centrifuged at 800 x *g* for 10 min followed by centrifugation of the supernatant at 2000 x *g* for 5min and the obtained mitochondrial fraction was used for the following estimations (30, 31).

Lipid peroxidase (LPO) activity: LPO product malondialdehyde (MDA) was estimated using 1, 1, 3, 3-tetraethoxypropane as the standard and is expressed as nmol/mg protein (32).

Superoxide dismutase (SOD) activity: SOD was estimated by following the procedure of (33). The inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored formozan in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. One unit (U) of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50% in 1 min under the defined assay conditions and the results have been expressed as U/mg protein.

Catalase (CAT) activity: Decomposition of H_2O_2 in presence of catalase was followed at 240 nm (34). One unit of (U) CAT was defined as the amount of enzyme required to decompose 1 mmol of H_2O_2/min, at 25^oC and pH 7.0. Results are expressed as U/mg protein.

HCl/EtOH Induced Gastric Ulcers

The animals were divided into six groups each consisting of six rats. Group 1 represented normal control (non-ulcerated) animals received suspension of 1% CMC in distilled water. Group 2 represented as control animals received suspension of 1% CMC in distilled water. Group 3 received Carbenoxolone (200 mg/ kg). Groups 4–6, 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, for 48 h fasted rats the last dose was administered, 60 min prior to induction of gastric ulcers by oral administration of 1.0 ml HCl/EtOH (60 ml EtOH+1.7 ml HCl+38.3 ml H_2O) (35). The animals were sacrificed and examined for gastric ulcers 60 min later. Ulcer index was scored as described earlier (18).

Cysteamine-Induced Duodenal Ulcers

The animals were divided into five groups each consisting of six mice. Group 1 represented control group of animals received suspension of 1% CMC in distilled water Group 2 received Omeprazole (14 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. Acute duodenal lesions were induced in mice according to the method of Selye (36). Three doses of cysteamine HCl (350 mg/kg) in distilled water were administered orally by a 3.5h interval. A test drugs was orally given 30 minutes prior to each dose of cysteamine. All animals were killed 24 h after the first dose of cysteamine and the duodenal lesions was examined. The lesion index was scored as described earlier (18).

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.

Pyloric Ligation Induced Ulcer

The effect of PHF at various doses was studied in pylorus ligated gastric ulcer model in rats. PHF at 100, 200 and 400 mg/kg given orally, once daily for three days showed dose dependent protective effect against gastric ulcer induced by pyloric ligation. PHF at a doses of 200 and 400 mg /kg inhibited ulcer formation significantly ($P<0.001$), but failed to do so for at 100mg/kg. Omeprazole, the reference antiulcer agent, significantly ($P<0.001$) inhibited the ulceration induced by pyloric ligation. The gastric volume and pH were 11.55 ± 0.27 ml and 1.31± 0.98 respectively, in the vehicle treated rats. As shown in Table 2, the PHF decreased dose dependently the volume of gastric juice. The effects were remarkable at doses of 200 and 400 mg/kg as compared to those in the vehicle treated groups. The pH value was also increased dose dependently by the treatment with the PHF.

Table 2: Effect of polyherbal formulation (PHF mg/kg, for 3 days) on Ulcer index, gastric volume, pH, and DNA in 4 h pylorus ligated rats

Drug treatment	Ulcer Index	% Protection	Gastric volume (ml)	pH	DNA (µg/ml)
Control	72.33 ± 3.40	--	11.55 ± 0.27	1.31± 0.98	228.80 ± 3.12
Omeprazole (10 mg /kg)	3.83 ± 0.65***	94.70	3.95 ± 0.08***	3.68 ± 0.13***	109.78 ± 2.97***
PHF 100	71.17 ± 2.52	1.79	11.37 ± 0.29	1.75 ± 0.12	214.28 ± 3.84*
PHF 200	34.00 ± 3.51***	53.08	7.85 ± 0.21***	2.02 ± 0.15***	181.18 ± 5.26***
PHF 400	8.17 ± 0.48***	88.73	4.30 ± 0.18***	3.02 ± 0.26***	119.82 ± 6.57***

Values are presented as mean ± SEM (n = 6); ***$P<0.001$ Vs control: **$P<0.01$ Vs control: *$P<0.05$ Vs control

The amount of DNA present in the gastric juice after the test drug treatment is an indication of increase or decrease in life span of mucosal cells. PHF at 200 and 400 mg/kg decreased the DNA content of the gastric juice significantly ($P<0.001$) and 100 mg/kg decreased the DNA content less significantly ($P<0.05$). The above result indicates that there was decrease in cell shedding.

The values of free acid, total acid, protein and dissolved muco substances were given in Table 3. When compare to control, there was significant ($P<0.01$, $P<0.001$) decrease in free acid at 200 and 400mg/kg, respectively. All the three doses of PHF significantly ($P<0.001$) reduced total acid and PHF at 200 and 400 mg/kg decreased the protein content of the gastric juice significantly ($P<0.001$) and 100 mg/kg decreased the protein content less significantly ($P<0.01$). The PHF 200 and 400mg/kg reduced the levels of pepsin significantly ($P<0.001$).

PHF was also studied for its effect on dissolved muco substances of gastric juice. It showed significant ($P<0.001$) rise in total Hexose, Fucose and Sialic acid at 200 and 400 mg/kg. 100 mg/kg of PHF increase the level of Fucose significantly ($P<0.05$) when compare to control. PHF at 400 mg/kg increased Hexosamine significantly ($P<0.001$) and 200 mg/kg increased it less significantly ($P<0.01$).

The effect of PHF on cell proliferation was shown in Table 4. The effect of PHF was studied on both the weight of the glandular portion of rat stomach and µg DNA/mg protein, which are indicative of any cell proliferation. When compare to control animals, PHF 400mg/kg showed less significant ($P<0.05$) change in mucosal protein and glandular weight of rat stomach. There was no significant change in DNA and µg DNA/mg protein. PHF did not show any significant change in above parameters.

Indomethacin induced ulcer

Effect of PHF on ulcer index and % protection in indomethacin (30mg/kg) induced ulcer in rats were shown in Table 5. Administration of indomethacin resulted in the production of gastric lesions mainly in the glandular portion of the stomach. The rats treated with PHF significantly ($P<0.001$) decreased the intensity of gastric mucosal damage induced by indomethacin. The % protection of gastric lesion was more (69.63%) in the groups of animals received PHF 400 mg/kg when compare to PHF 100 and 200mg/kg.

Table 3: Effect of polyherbal formulation (PHF mg/kg, for 3 days) on gastric juice mucoprotein in 4 h PL rats

Drug treatment	Free Acid (µgeq/100gm)	Total acid (µgeq/100gm)	Pepsin (µg/ml)	Protein (µg/ml)	Total Hexose (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Sialic acid (µg/ml)	Total Carbohydrate (µg/ml)	TC:P
Control	214.08 ± 5.65	340.97 ± 6.04	49.35 ± 1.35	346.75 ± 13.45	285.43 ± 6.37	161.27 ± 1.38	55.42 ± 1.87	32.23 ± 0.55	534.35 ± 4.87	1.55 ± 0.06
Omeprazole (10 mg /kg)	106.02 ± 3.19***	217.00 ± 2.85***	12.88 ± 1.43***	170.65 ± 6.56***	456.62 ± 4.67***	192.78 ± 4.39***	72.68 ± 1.21***	49.68 ± 0.56***	771.35 ± 3.65***	4.56 ± 0.18***
PHF 100	203.80 ± 3.83	311.30 ± 2.20***	47.47 ± 2.36	288.43 ± 22.14**	289.10 ± 8.14	162.27 ± 2.27	60.82 ± 0.91**	34.73 ± 0.69	546.92 ± 9.37	1.96 ± 0.17*
PHF 200	195.55 ± 2.96**	293.75 ± 3.23***	38.40 ± 0.91***	187.52 ± 6.53***	411.68 ± 3.74***	179.32 ± 5.60**	71.02 ± 0.99***	43.28 ± 1.54***	705.30 ± 4.77***	3.78 ± 0.13***
PHF 400	109.65 ± 4.42***	259.45 ± 6.54***	20.00 ± 0.92***	176.70 ± 2.18***	444.63 ± 3.54***	181.35 ± 3.33***	72.55 ± 1.16***	49.48 ± 1.94***	748.02 ± 5.59***	4.24 ± 0.07***

Values are presented as mean ± SEM (n = 6); ***P<0.001 Vs control: **P<0.01 Vs control: *P<0.05 Vs control

Table 4: Effect of polyherbal formulation (PHF mg/kg, for 3 days) on cell proliferation and weight of glandular portion of stomach in 4 h PL rats

Drug treatment	Weight of glandular portion of stomach (mg/100 g BW)	Cell Proliferation		
		Protein	DNA µg/100 mg wet tissue	µgDNA/mg Protein
Control	441.83 ± 4.25	5568.5± 23.65	568.0 ± 2.67	103.17 ± 1.70
Omeprazole (10 mg /kg)	484.33 ± 4.27***	5891.3 ±23.33***	605.83 ± 1.38***	95.33 ± 1.49***
PHF 100	448.67 ± 3.32	5602.7 ± 13.17	568.50 ± 2.57	104.0 ± 0.58
PHF 200	450.17 ± 0.48	5614.2 ± 1.45	570.0 ± 0.58	102.83 ± 0.31
PHF 400	451.00 ± 0.58*	5616.8 ± 0.79*	573.33 ± 1.02	102.17 ± 1.99

Values are presented as mean ± SEM (n = 6);
***P<0.001 Vs control: **P<0.01 Vs control: *P<0.05 Vs control

Table. 5. Effect of poly herbal formulation (PHF mg/kg, for 3 days) on ulcer index on indomethacin induced ulcer in rats

Drug treatment	Control	Omeprazole (10mg/kg)	PHF 100	PHF 200	PHF 400
Ulcer index	18.67 ± 1.12	3.17 ±0.60 ***	12.00 ± 0.58 ***	7.50 ± 0.67 ***	5.67 ± 0.49 ***
% Protection	--	83.02	33.73	59.83	69.63

Values are presented as mean ± SEM (n = 6)
***P<0.001 Vs control: **P<0.01 Vs control: * P<0.05 Vs control

Stress induced ulcer

The Effect of polyherbal formulation on ulcer index, LPO, SOD and CAT in cold restraint stress ulcer was shown in Table 6. Hypothermic and Immobilization stress produced considerable ulcerogenicity in rats. The ulcers were in the form of hemorrhagic mucosal lesions in the stomach, which were confined to the rugae of glandular segment. The parameters studied included ulcer index and % protection of ulcer by PHF. PHF at the doses of 100, 200 and 400 mg/kg resulted in a significant (P<0.001) reduction in ulcer index (34.83 ± 5.29, 12.33 ± 1.15and 9.33 ± 0.99 respectively) and % protection of ulcer (52.63, 83.23 and 87.31 respectively) when compared with the control group. Standard drug showed significant anti-ulcer activity in this model.

Stress significantly increased lipid per oxidation in the gastric mucosa with concomitant increase in SOD and decrease in CAT. PHF in all the three doses of 100, 200 and 400 mg/kg significantly protected the animals against stress-induced free radical damage as seen from the decrease in LPO and reversal of changes induced by stress on SOD and CAT.

Table 6: Effect of polyherbal formulation (PHF mg/kg, for 3 days) on ulcer index, LPO, SOD and CAT in cold restraint stress ulcer model in rats

Drug treatment	Ulcer Index	% Protection	LPO (nmols of MDA/mg of protein)	SOD (U/mg of protein)	CAT (U/mg of protein)
Normal Control	--	--	134.05 ± 11.75***	94.57 ± 1.59***	55.33 ± 2.88***
Stress Control	73.67 ± 2.11	--	242.92 ± 7.99	149.70 ± 3.10	20.40 ± 1.58
Omeprazole (10 mg /kg)	5.67 ± 0.49***	92.29	141.80 ± 2.58***	111.92 ± 3.39***	46.87 ± 2.04***
PHF 100	34.83 ± 5.29***	52.63	204.42 ± 5.04***	135.55 ± 1.53**	22.47 ± 1.49
PHF 200	12.33 ± 1.15***	83.23	157.92 ± 7.26***	117.88 ± 4.42***	27.18 ± 1.91*
PHF 400	9.33 ± 0.99***	87.31	152.03 ± 2.70***	107.47 ± 4.26***	45.95 ± 1.29***

Values are presented as mean ± SEM (n = 6)
***P<0.001 Vs control: **P<0.01 Vs control: *P<0.05 Vs control

HCl/EtOH-induced gastric ulcers

Effect of poly herbal formulation on ulcer index, Hexosamine and gastric wall mucus on HCl/EtOH induced ulcer in rats was given in Table.7. In HCl/EtOH-induced gastric ulcers, the lesions were characterized by multiple-hemorrhage red bands of different sizes along the long axis of the glandular stomach. The results observed with PHF at 100, 200 and 400 mg/kg demonstrated significant inhibition of ulcerative lesion by 64.49%, 90.46% and 96.18%, respectively, as compared to the control value. Carbenoxolone, the standard drug produced 92.85% inhibition of ulcer.

PHF at doses of 100, 200 and 400 mg/kg significantly restored the mucus content back to a level comparable to that for the non-ulcerated rats. The mucus content in ulcerated group was 812.47±41.54 as compared to PHF at 100, 200 and 400 mg/kg treated (997.82±14.32, 1042.5±20.73 and 1196.8±13.17 respectively) groups. Also in the assay for gastric hexosamine content, the mean gastric Hexosamine content in control ulcerated rats was significantly less than that in the normal non-ulcerated group, as shown in Table. 6.

Pretreatment with PHF at 100, 200 and 400 mg/kg increased the hexosamine content significantly (28.17 ± 0.71, 32.35 ±1.18 and 36.13 ± 0.43µmg/100 mg wet stomach, respectively, compared to 20.48 ± 1.22 µg/100 mg wet stomach for the control HCl/EtOH ulcerated rats.

Table. 7. Effect of poly herbal formulation (PHF mg/kg, for 3 days) on ulcer index, Hexosamine and gastric wall mucus on HCl/EtOH induced ulcer in rats

Drug treatment	Ulcer Index	% Protection	Hexosamine (µg/ 100 mg wet stomach)	Gastric wall mucus (µg/Alcian blue/g wet stomach)
Normal Control	--	--	38.03 ± 0.39***	1166.3 ± 9.52***
Stress Control	69.83 ± 2.89	--	20.48 ± 1.22	812.47 ± 41.54
Carbenoxolone (200 mg /kg)	5.00 ± 0.58***	92.85	34.98 ± 0.68***	1064.0 ± 20.34***
PHF 100	24.83 ± 3.79***	64.49	28.17 ± 0.71***	997.82 ± 14.32***
PHF 200	6.67 ± 0.42***	90.46	32.35 ± 1.18***	1042.5 ± 20.73***
PHF 400	2.67 ± 0.67***	96.18	36.13 ± 0.43***	1196.8 ± 13.17***

Values are presented as mean ± SEM (n = 6)
***P<0.001 Vs control: **P<0.01 Vs control: * P<0.05 Vs control

Cysteamine induced duodenal ulcer

PHF was evaluated for anti-ulcerogeinc activity in the duodenal ulcer induced by cysteamine administration in mice and given in Table.8. The parameters studied in cysteamine induced duodenal ulcer were ulcer index and % protection of ulcer by PHF. PHF at the doses of 100, 200 and 400 mg/kg resulted in a significant (P<0.001) reduction in ulcer index (5.83 ± 0.48, 3.67 ± 0.33 and 2.67 ± 0.33 respectively) and % protection of ulcer (52.72, 70.24 and 78.35%. respectively) of duodenal lesions when compared with the control group. Standard drug showed significant anti-ulcer activity in this model.

Table. 8. Effect of poly herbal formulation (PHF mg/kg, for 3 days) on ulcer index on Cysteamine induced duodenal ulcer in mice

Drug treatment	Control	Omeprazole (14mg/kg)	PHF 100	PHF 200	PHF 400
Ulcer index	12.33 ± 1.12	1.83 ± 0.31***	5.83 ± 0.48***	3.67 ± 0.33***	2.67 ± 0.33***
% Protection	--	85.16	52.72	70.24	78.35

Values are presented as mean ± SEM (n = 6)
***P<0.001 Vs control: **P<0.01 Vs control: * P<0.05 Vs control

Discussion

PHF showed significant ulcer protective effects as observed from significant decrease in acute ulcers induced by pyloric ligation and indomethacin. Ulcers due to pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa (37). Ulcers caused by indomethacin are due to decrease in PG synthesis and increase in acid secretion (37). Multiplicity of factors responsible for peptic ulcer makes difficult to pinpoint its exact etiology. Nevertheless, it is clear that it results from an imbalance between offensive and defensive factors. Naturally occurring substances have been found to possess anti ulcerogeinc activity by lowering offensive factors of augmenting defensive factors for ulcerogenesis (37). The efficacy of PHF as anti ulcerogeinc could be due to its various actions on offensive and defensive factors. Hence further investigation carried on offensive and defensive factors in the gastric juice and mucosa.

Increased in offensive factors have been reported to be essential for many experimental and clinical gastric ulcers (38) PHF significantly decreased the acid and pepsin and increased the defensive mucin secretion quantified in terms of TC:P ratio of the gastric juice. Mucin is viscous glycoprotein with physiochemical properties producing relatively resistant acid barrier (39). It makes up the major part of the mucus an important pre epithelial factor that act as a first line of defense against ulcerogens (40) . Increase mucin was due to significant increase in individual muco polysaccharide like Sialic acid and total hexoses leading to significant increase in total carbohydrates. PHF significantly increased glycoprotein content of mucosal cells as seen from the increase in the TC: P ratio of gastric mucosa. This shows that PHF induces turn over of glycoprotein in the mucosal cells, thus increasing the quantity of cellular mucous. The increase in mucosal defense is further exemplified by decrease in cell exfoliation as seen from the decrease in DNA content of the gastric juice (27). Cell proliferation is not affected by PHF, because it did not reduce any significant changes of DNA content in the gastric mucosa and its glandular weight. Hence PHF may maintain integrity of gastric mucosa could involve the process of restitution rather than cell proliferation. Restitution is a process of movement of viable mucosal cells to cover the injured mucosa (41).

According to the experimental models used in the study NSAIDs by indomethacin induced ulcer formation by depletion cyto-protective PGs. PGE_2 and PGI_2 of gastric and duodenal mucosa are responsible so mucous production and maintaining cellular integrity of gastric mucosa (42).

PHF caused a significant inhibition in stress-induced gastric ulcer. Regarding the cold and restraint stress model, it has been reported that stress-induced gastric lesions develop as a result of multifactorial impairment of mucosal defense system, disturbance of gastric mucosal microcirculation (43), stimulation of vagal nerve, which increases gastric secretion (44) and gastric motility (45). Apart from peripheral events, central mechanisms including vagal over activity have also been considered for the pathogenesis of stress ulcers (46, 47). Based on the results of this study, it could be suggested that inhibition of acid hyper secretion, increase in gastric mucus secretion or alterations in gastric mucosal blood flow, might be involved in the protection afforded by PHF in this model.

The role of free radicals in gastric ulcerations is well documented (48). PHF significantly reduced lipid per oxidation in rat gastric mucosa. SOD scavenges the super oxide radical O_2-, one of the reactive oxygen species (ROS) responsible for lipid per oxidation (49). This reaction leads to increase in generation of peroxyl radical H_2O_2-, which is also capable of producing more oxidative damage (50). CAT and other peroxidases further reduce H_2O_2-. Hence, the anti-oxidant activity in gastric mucosal homogenates observed from decrease in LPO may be due to increase in SOD and CAT levels. Stress-induced ulceration involves damage by ROS apart from acid and pepsin related factors (51). During stress LPO and SOD were significantly increased and CAT level was significantly decreased. The increase in SOD was due to increased ROS generation during mucosal damage. This led to increased generation of H_2O_2- and its accumulation due to decreased CAT level. Inactivation of gastric peroximes during stress (52) may also aggravate the mucosal damage. This evidently caused increased lipid per oxidation and mucosal damage as seen from the increase in ulcer index in comparison to the control group. PHF effectively alleviated stress-induced ulcers with marked decrease in LPO, suggesting decrease in oxidative damage. This may be due to restoration of balance between free radical scavenging enzymes SOD and CAT in the gastric mucosa, effectively counteracting the free radicals generated by cascade of reactions as described earlier. Thus, the anti-ulcerogeinc activity of PHF may also be due its anti-oxidant effects.

The ability of the gastric mucosa to resist injury by endogenous secretions (acid, pepsin and bile) and by ingested irritants (e.g., alcohol), can be attributed to a number of factors that have been referred to collectively as mucosal defense (53). The formation of gastric mucosal lesions by necrotizing agents such as HCl and EtOH has been reported to involve the depression of these gastric defensive mechanisms (54). HCl/EtOH-induced gastric ulcers also promote stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissue injury (55). In the HCl/Ethanol induced gastric ulceration model HCl causes severe damage to gastric

mucosa (56) whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus (57). EtOH-induced ulcers are not inhibited by anti-secretary agents but are inhibited by agents that enhance mucosal defensive factors such as prostaglandins (58). These results show that PHF probably have an anti-ulcerogeinc effect related to cyto-protective activity, as the extract presented significant results in the ethanol model.

Gastric wall mucus, an obligatory component of which is hexosamines, is thought to play an important role as a defensive factor against gastrointestinal damage (59). The determined gastric wall mucus was used as an indicator for gastric mucus secretion, while mucosal hexosamine content was used as an indicator for gastric wall mucus synthesis (60). In the present study, gastric wall mucus and hexosamine contents in HCl/EtOH ulcerated rats were markedly lowered as compared to those of the non-ulcerated group. It was found that pretreatment with PHF increased both gastric mucus and Hexosamine contents significantly in HCl/EtOH-ulcerated rats. This finding indicates that the PHF preserves both gastric mucus synthesis and secretion in the experimental rats.

Cysteamine-induced duodenal ulcer in the mice is widely used as a model of peptic ulcer disease. This chemically induced ulcer resembles the duodenal ulcer in man (36). Cysteamine induces long-lasting hyper secretion of gastric acid, which may be partly due to increased plasma levels of gastrin. In addition, cysteamine inhibits secretion of alkaline mucus from the duodenal Brunner's gland. Hyper-secretion of acid, disturbed gastro duodenal motility, hyper-gastrinemia and decreased mucosal resistance has all been implicated in the pathogenesis of duodenal ulcer disease in man (61). The development of duodenal ulcers in response to cysteamine is inhibited by anti-cholinergic agents, antacids, prostaglandins and histamine H_2-receptor antagonists (62). Therefore, the inhibition of gastric acid secretion and mucus and prostaglandin release and/or gastric acid neutralization and a mechanical barrier occurring in the duodenum may interfere with duodenal mucosa protection (63).The anti-ulcer activity of PHF on cysteamine induced duodenal ulcer may be due to anti-secretary and enhanced mucus production.

In conclusion, the study provides evidence that the polyherbal formulation possesses anti-ulcer activity which may be due to anti-secretary, enhanced production and restoration of mucus and partly by scavenging the free radicals generation.

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[#] For correspondence

S.Sengottuvelu.,
Assistant Professor,
Department of Pharmacology,
Nandha College of Pharmacy,
Perundurai main road,
Erode-638 052,
Tamilnadu. India
E-mail: sengt@rediffmail.com