

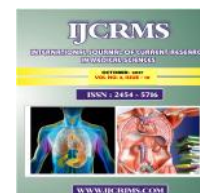


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Antiulcer activity of Muppirandai chooranam against Pylorus Ligated (SHAY) Rat ulcer model

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Abstract

Siddha poly herbo formulation *Muppirandai chooranam* indicated as a best choice of drug to treat Peptic ulcer disease. MC was evaluated for its anti ulcer activity on Pyloric Ligation method Induced Ulcer in rats. At the end of the results, can found that the drug MC has anti ulcer activity effect. Oral administration of MC (360mg/kg), significantly reduced the elevated peptic ulcer disease levels as the duration of drug administration increases. Further clinical study will be carried out for the benefit of peptic ulcer disease patients.

Keywords: *Muppirandai chooranam*, Peptic ulcer, MC, anti ulcer activity.

Introduction

Siddha system is a unique system which is highly incorporated with science and spirituality. Muppirandai(*Cissus quadrangularis* three sided) is one of the drug which is mentioned in siddha literatures for various diseases especially for Peptic ulcer disease. Herbal and poly herbal preparations are being considered as good in nature because of its therapeutic value. Characterization is also essential to know the

structural and functional property of herbal formulation for wide use.

Experimental Section

Details regarding the sample

Muppirandai chooranam is a siddha poly herbal formulation which is indicated as a drug in siddha

sastric text. “yaekoebu vaithiya chindhamani 700” for the treatment of peptic ulcer disease, ascites, jaundice, edema, menorrhagia, renal calculi, cardiac disorders etc.,. The ingredients of Muppirandai chooranam are five in number. They are *Cissus quadrangularis* (three sided) *Trachyspermum ammi*, *Piper nigrum*, *Piper longum*, *Zingiber officinale* (dried). The drug was prepared as per the text.

Animals

Male Wistar albino rats weighing 180 – 200 gms were used for the study. The animals were obtained from animal house, Nandha College of Pharmacy, Erode. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a standard environmental condition (Humidity of 30 – 70 % and 12:12 light: dark cycle at 24±2°C). 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/PO/Re/S/02/CPCSEA) and were in accordance with the Institutional ethical guidelines.

Pyloric Ligation Induced Ulcer

The method of Shay rat ulcer was adopted (Shay et al., 1945). The animals were divided into three groups of six each. Group I, control animals received 1ml/kg of 0.1% Carboxymethyl Cellulose (CMC) solution. Group II received Omeprazole (10 mg/kg) and Groups III received Muppirandai Chooranam (360mg/kg). The test drugs were administered for three days, orally by suspending in 0.1% 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., 1976).

- 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.

Biochemical Parameters investigated in gastric juice

Estimation of dissolved mucosubstances is done by determining the total carbohydrates and protein in 95% ethanol precipitate of gastric juice. From gastric juices, the following parameters like gastric volume, pH, free acidity, total acidity, pepsin activity, total carbohydrates (Hexose, Hexosamine, Fucose and Sialic acid), protein content and total carbohydrate/protein ratio were determined.

Gastric Juice Volume and pH

The volume and pH of centrifuged gastric juice were measured by measuring cylinder and digital pH meter.

Total and Free Acidity

Pipette 1ml of filtered gastric contents into a small beaker, add 2 to 3 drops of Topfer’s reagent or methyl orange and titrate with 0.01 N NaOH until all trace of the red colour disappears and the colour is yellowish orange. Note the volume of alkali added that indicate free acidity. Then add 2 or 3 drops of phenolphthalein and continue

titrating until a definite red tinge reappears. Note the total volume of alkali added that indicate total acidity. The results expressed as mEq/litre.

Pepsin Activity

Centrifuged gastric juice of 0.1 ml ($5000 \times g$ for 10 minutes) was added to 1 ml of bovine albumin (0.5% w/v in 0.01 N HCl, pH 2) and incubated for 20 minutes at 37°C. A duplicate background control tube (gastric juice blank) in which 1 ml albumin was replaced with 1 ml of 0.01 N HCl was run simultaneously. The hydrolysis was stopped by adding 2 ml of 10% trichloroacetic acid. All tubes were heated in boiling water for 5 minutes and cooled. After denaturation of the protein by heating in a boiling water bath for 5 minutes, the precipitate was removed by centrifugation ($9000 \times g$ for 10 minutes). A total of 1 ml of the supernatant was mixed with 0.4 ml of 2.5 N NaOH and 0.1 ml of the Folin-Ciocalteu reagent and the volume was adjusted to 10 ml with distilled water. The absorbance was measured at 700 nm. The peptic activity was calculated in terms of micrograms of tyrosine liberated per milliliter of gastric juice.

Total Carbohydrates

Hexose

Hexose level was estimated by the method of Niebes. 0.5 ml of the neutralized solution was made upto 1 ml with distilled water and added 8.5 ml ice-cold orcinol reagent. The mixture was heated at 80°C for 15 minutes, cooled and left in the dark for 25 minutes for colour development. Then absorbance was read at 540 nm in a colorimeter. The hexose content was expressed as µg/ml.

Hexosamine

Hexosamine content was estimated by the method of Wagner. 0.5 ml of the neutralized sample was made upto 1 ml with distilled water. Standard galactosamine was also made upto 1 ml with distilled water. 0.6 ml of acetyl acetone reagent was added to all the tubes and heated in a boiling water bath for 30 minutes. After cooling, 2 ml of

Ehrlich's reagent was added and the contents were shaken well. The pink colour developed was measured at 540 nm against the reagent blank. Hexosamine content was expressed as µg/ml.

Fucose

Fucose level was estimated by the method of Dische and Shettles. 0.05 ml of the neutralized sample and 5 ml of sulphuric acid: water mixture was added and heated in a boiling water bath for 10 minutes. After cooling the tubes, 0.1 ml of cysteine reagent was added. The colour developed after 150 minutes was read at 420 nm. The standard was also treated in a similar manner. The fucose level was expressed as µg/ml.

Sialic Acid

Sialic acid level was determined by the method of Warren. 0.5 ml of the neutralized sample was taken along with the standards. Blank contained 0.5 ml of 0.1 N sulphuric acids. 0.25 ml of periodate was added to all tubes at 37°C. After 30 minutes, 0.25 ml of arsenite solution was added to inhibit the reaction. Contents were mixed well and 2 ml of thiobarbituric acid was added and the tubes were heated in a boiling water bath for 6 minutes. After cooling, the pink colour developed was extracted into 5 ml of acidified butanol phase and was measured at 540 nm against a reagent blank. The sialic acid content was expressed as µg/ml.

Protein Content

Estimation of protein was carried out as described by Lowry in 1951. One milliliter of gastric juice and 9 ml of 95% alcohol was mixed, shaken, and then the mixture was centrifuged at $3000 \times g$ for 15 minutes to obtain the precipitation. This precipitate was dissolved in 1 ml of 0.1 N NaOH. Next 0.9 ml of distilled water was added to 0.1 ml of the above-mentioned solution. Out of this solution, 0.4 ml was taken in another test tube. Four milliliters of alkaline reagent was added to this test tube and kept for 10 minutes. Then 0.4 ml of phenol reagent was added to this test tube and kept for 10 minutes for color development. The readings were taken against the blank prepared

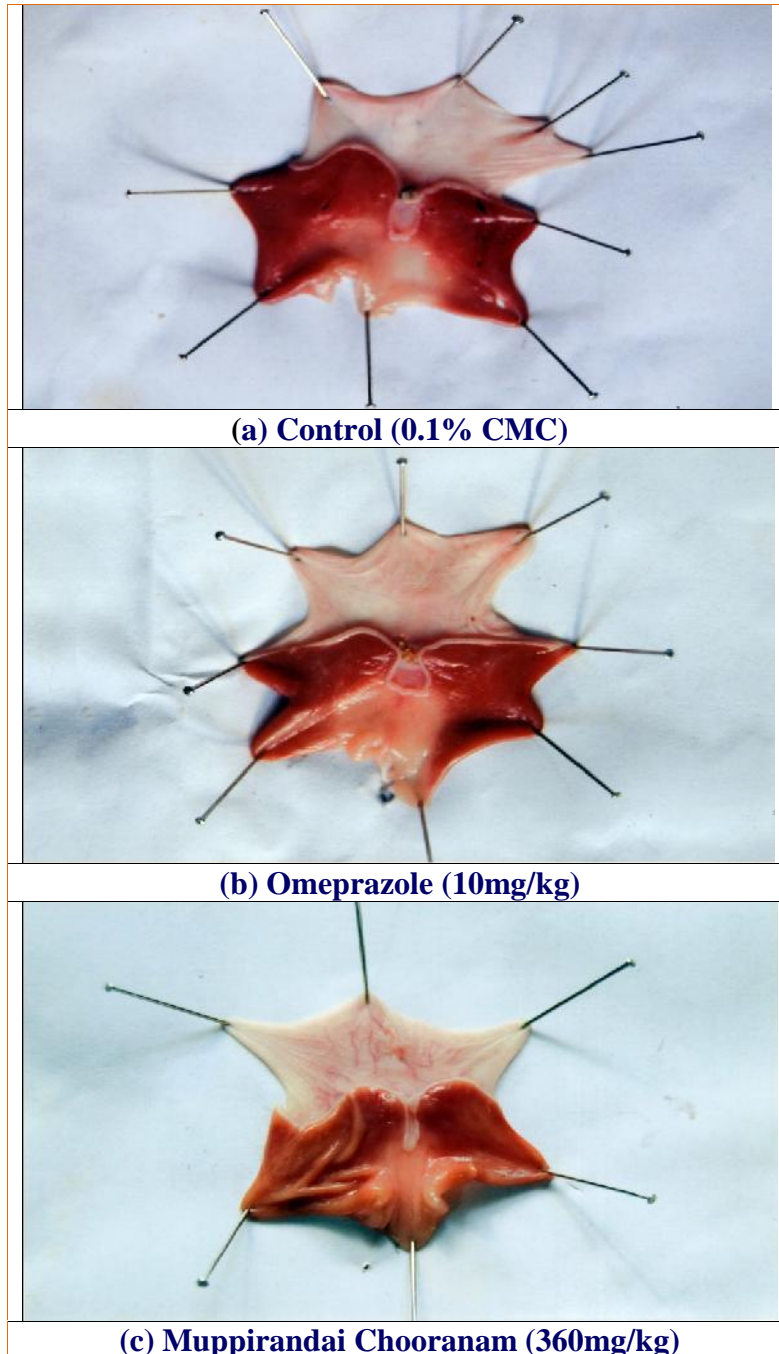
with distilled water. The protein content was obtained by calculating with the use of standard curve prepared with bovine albumin. The concentrations of proteins were expressed in terms of micrograms per milliliter of gastric juice.

Statistical Analysis

Results were expressed as mean \pm SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test using GraphPad version 3. P values < 0.05 were considered as significant.

Results

Photo 1. Effect of Muppirandai Chooranam on Pylorus Ligated Ulcer Model in Rats



The effect of Muppirandai Chooranam (360mg/kg) was studied in pylorus ligated gastric ulcer model in rats. Table 1. shows the effect Effect of Muppirandai Chooranam on Ulcer Index, Gastric Volume and pH of Pylorus Ligated ulcer in rats. in pylorus ligated rat the gastric volume was 10.64±0.85 ml and it was significantly (P<0.001) decreased by both Omeprazole and MC to 3.88±0.14 and 4.59±0.22

ml respectively. The pH of gastric volume in control was 1.45±0.05 and it was significantly increased to 3.66±0.11 in Omeprazole treated groups but there was no significant change in the animals treated with MC. Ulcer index in the control animals was 51.45± 2.67 and it was significantly (P<0.001) decreased by both Omeprazole and MC to 9.36 ±0.25 and 12.46 ± 0.98 respectively.

Table 1. Effect of Muppirandai Chooranam on Ulcer Index, Gastric Volume and pH of Pylorus Ligated ulcer in rats.

Drug Treatment	Ulcer Index	% Protection	Gastric Volume (ml)	pH
Control 0.1 % CMC (1ml/kg)	51.45± 2.67	-	10.64± 0.85	1.45± 0.05
Standard Control Omeprazole (10mg/kg)	9.36± 0.25***	81.81	3.88± 0.14***	3.66± 0.11*
MC (360 mg/kg)	12.46± 0.98***	75.78	4.59± 0.22***	2.47± 0.16

Values are in mean ± SEM (n=6),

*P<0.05 , **P<0.01, ***P<0.001 Vs Control

Table 2. Effect of Muppirandai Chooranam on Free Acidity, Total Acidity and Pepsin of Pylorus Ligated ulcer in rats. Free acidity of control animals shows 85.74± 6.62 and it was significantly decreased by Omeprazole (P<0.001) and MC (P<0.01) to 44.66±2.70 and 58.05±4.32 respectively. Total acidity of control animals

shows 105.74± 5.22 and it was significantly decreased by Omeprazole (P<0.001) and MC (P<0.01) to 64.66±4.75 and 76.05±3.79 respectively. Similarly the pepsin activity also significantly decreased by Omeprazole (P<0.001) and MC (P<0.01) to 8.74±0.66 and 25.63±0.95 respectively compared to control.

Table 2. Effect of Muppirandai Chooranam on Free Acidity, Total Acidity and Pepsin of Pylorus Ligated ulcer in rats.

Drug Treatment	Free Acidity (mEq/Lit)	Total Acidity (mEq/Lit)	Pepsin Activity (U/ml)
Control 0.1 % CMC (1ml/kg)	85.74± 6.62	105.74± 5.22	42.22± 2.90
Standard Control Omeprazole (10mg/kg)	44.66± 2.70***	64.66± 4.75***	8.74± 0.66***
MC (360 mg/kg)	58.05± 4.32**	76.05± 3.79**	25.63± 0.95**

Values are in mean ± SEM (n=6),

*P<0.05 , **P<0.01, ***P<0.001 Vs Control

MC was also studied for its effect on dissolved muco substances in gastric juice and the results were shown on Table 3. It showed marked raise in total Hexose and Sialic acid compared to control significantly ($P<0.01$). It also significantly

($P<0.05$) raised the Hexosamine, Fucose and Protein compared to control groups. Omeprazole and MC were reduced the TC:P ratio compared to control

Table 3. Effect of Muppirandai Chooranam on Mucoproteins (Hexose, Hexosamine, Fucose, Sialic Acid and Protein) of Pylorus Ligated ulcer in rats.

Drug Treatment	Hexose (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Sialic Acid (µg/ml)	Total Carbohydrate (TC) (µg/ml)	Protein (P) (µg/ml)	TC:P
Control 0.1 % CMC (1ml/kg)	277.82± 8.76	145.39± 6.90	52.50± 2.95	28.63± 1.17	504.34± 15.58	433.31± 20.06	1.13
Standard Control Omeprazole (10mg/kg)	462.79± 11.45***	190.43± 7.15**	78.31± 3.33*	51.26± 2.32***	782.79± 21.26**	355.56± 16.87**	2.01
MC (360 mg/kg)	416.52± 9.43**	164.74± 6.33*	70.44± 5.62*	48.42± 2.45**	700.12± 18.72**	379.72± 15.44*	1.84

Values are in mean ± SEM (n=6),

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ Vs Control

Discussion

Mucopolysaccharides such as hexose, hexosamine, fucose, sialic acid are seen in the fluids which can be secreted after the ingestion of this medicine. These may be the responsible factors to heal peptic ulcers. Mucopolysaccharide metabolism during the varying stages of gastric ulcer, formation of a mucous barrier is quite rapidly initiated after ulceration.

Conclusion

Omeprazole causes many adverse effects. But this *Muppirandai Chooranam* will never cause any adverse effects. It is very good appetizer. So it enhance the digestive capacity and heal the peptic ulcer disease. These results suggest that the gastric mucosal hexosamine, hexose, fucose, sialic acid and protein is closely related to the onset and healing of Pyloric Ligation method Induced Ulcer in rats to prevention and healing of ulcers by increasing gastric mucopolysaccharides.

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