Antiulcer Activity of Siddha Herbomineral Formulation

Menilavana Chooranam against Aspirin plus Pylorus Ligation Induced Ulcer in Rats

Keywords: Antiulcer, Menilavana Chooranam, Siddha, herbomineral formulation, aspirin plus pylorus ligation induced ulcer

ABSTRACT

Siddha system of medicare is holistic with more of literature and experiences. A great number of formulations are described in Siddha literature for treatment of peptic ulcer. The ingredients of Menilavana Chooranam are widely used in Siddha system to cure various symptoms of peptic ulcer. To investigate the antiulcer activity of Menilavana Chooranam against aspirin plus pylorus ligation induced ulcer in rats. Acute oral toxicity study was carried out in normal female Wistar rats as per OECD guidelines 423. To study antiulcer activity Wistar rats were divided into five groups of six in each group. Aspirin was administered in non fasted rats once daily for eight days for all the groups except group I animals. Ranitidine (50 mg/kg) and Menilavana chooranam (200,400 mg/kg) were administered orally to the respective treatment groups 30 minutes before each Aspirin treatment, whereas the control group received only 1% SCMC and fasted for 18 hrs. On the ninth day, immediately after aspirin treatment, pylorus ligation was done under ether anesthesia. The stomach was dissected out after tying the esophageal end. The stomach contents were subjected to analysis for the acid secretary evaluation. Menilavana Chooranam showed significant reduction of gastric volume, gastric pH, free acidity, total acidity and gastric erosion. The results exhibited that Menilavana Chooranam possesses potent antiulcer activity against aspirin plus pylorus ligation induced ulcer in rats and might be useful in the treatment of peptic ulcer.
INTRODUCTION

The science of Medicine is one of the fundamental requirements for the survival and well-being of human race. Traditional system of medicare owed its origin and progress to great men whose tireless endeavours have not only enriched the medical science but also the society and civilization as a whole. This holistic system of medicare is the Siddha system. Modernization and consumerism have affected human health. Recurrent modification in lifestyle and diet leads to a lot of diseases. A proper diet is essential not only to maintain good health but also to prevent many diseases. Immorality in the dietary habits results in gastrointestinal disorders. All over the world, there is a proverb, “One man’s food is another man’s poison”. According to Siddha system, gastrium i.e., stomach is the main seat for several diseases, so there arises a need to take some steps for regulating and treating gastric disorders.

An ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. Ulcers occur within the stomach and/or duodenum is often chronic in nature. Burning epigastric pain exacerbated by fasting and improved with meals is symptom complex associated with peptic ulcer disease. Peptic ulcer disease encompasses both gastric and duodenal ulcer. Duodenal ulcer occurs most often in the first portion of duodenum (less than 95%), with 90 % located within 3 cm of the pylorus. Benign gastric ulcers are most found distal to the junction between the antrum and the acid secretory mucosa.

Our Siddha system is with more of literature and experiences. A great number of formulations are described in Siddha literature for treatment of peptic ulcer. The ingredients of *Menilavana Chooranam* are widely used in Siddha system to cure various symptoms of peptic ulcer. But the scientific society requires us to present our system scientifically to the world. To validate the medicine scientifically according to the modern parameters, *Meni Lavana Chooranam* was taken as the trial drug to investigate the antiulcer activity of against aspirin plus pylorus ligation induced ulcer in rats.

2. MATERIALS AND METHODS

2.1 Test drug: The Siddha herbomineral formulation *Menilavana Chooranam* has been selected from the classical Siddha literature, “Kannusamy Parambarai Vaithiyam” written by Maruthuvar C.Kannusamy Pillai.
2.2 **Ingredients:** Sukku (*Zingiber officinale*) - 500gm, Induppu (Sodium chloride impure)- 500gm, Kariuppu (Sodium chloride) - 500 gm, Omam (*Trachyspermum ammi*)- 500 gm, Perungayam (*Ferula asafoetida*) - 250 gm, *Acalypha indica* leaves.

2.3 **Collection of raw drugs:** The required raw drugs, Sodium chloride impure, Sodium chloride, dried *Zingiber officinale* rhizome, *Ferula asafoetida* and *Trachyspermum ammi* were collected from a raw drug shop at Chennai. *Acalypha indica* leaves were collected from Koyambedu, Chennai. They were identified and confirmed by the Head of the Gunapadam Department, Govt Siddha Medical College, Chennai.

2.4 **Purification of raw drugs:**

Sodium chloride impure: It was soaked in vinegar for 3 days and kept in sunlight for drying.

Sodium chloride: It was dissolved in water and filtered. The filtered solution was boiled for few minutes and dried in sunlight.

*Zingiber officinale*: The outer skin was peeled off and treated with Calcium carbonate for purification.

*Acalypha indica*: Leaves were washed well in running water to remove the impurities.

*Ferula asafoetida*: It was fried in ghee and purified.

*Trachyspermum ammi*: It was soaked in the limestone water for 3 hours and dried for purification.

2.5 **Preparation of Menilavana Choornam:**

The dried, scraped ginger rhizomes were taken in a vessel and soaked with the leaf juice of *Acalypha indica* for a day. Next day, this juice was poured out and ginger rhizomes were soaked with fresh *Acalypha indica* leaf juice. This process was repeated for 18 days. Then the rhizomes were dried in sunlight for 3 days. Induppu (sodium chloride impure), Kariuppu (sodium chloride) were grinded with *Acalypha indica* leaf juice and made into a paste. The ginger rhizome pieces (2-3 at a time) were pierced with a thin steel rod at its sharp end. It was immersed in the above salt paste and burnt till the salt started sparkling in the fire. *Trachyspermum ammi* and *Ferula asafoetida* were fried. All these were powdered, sieved and thus prepared drug *Menilavana choornam* was subjected to various studies.
FIG: 1 INGREDIENTS OF MENILAVANA CHOORANAM

Fig: 1-A. Sukku (*Zingiber officinale*)  
Fig: 1-B. Induppu (Sodium Chloride impura)

Fig: 1-C. Kariuppu (Sodium Chloride)  
Fig: 1- D. Omam (*Trachyspermum ammi*)

Fig: 1-E. Kuppaimeni (*Acalypha indica*)  
Fig: 1-F. Perumkayam (*Ferula asafetida*)
2.6 Experimental Animals:

Wistar rats of either sex weighing 150-200 gm were used. The animals were maintained in well-ventilated room, temperature was maintained at 27 ± 2°C with humidity 50 ± 5%. The animals were fed balance rodent pelleted diet from SAI DURGA feed, Bangalore and tap water ad libitum throughout the experimental period. The experimental protocol was approved by the IAEC (Ref.No 23/16 - CLBMCP dated 14.12.2006).

2.7 Acute oral toxicity study:

The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). The acute toxic class method is a stepwise procedure with 3 animals of a single sex per step. Depending on the mortality and/or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data based scientific conclusion. The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which causes acute toxicity.

2.8 Experimental procedure:

Female Wistar rats weighing 150–200 gm were used for the study. The starting dose level of the test drug was 2000 mg/kg body weight p.o. As most of the crude extracts possess LD_{50} value more than 2000 mg/kg p.o., the starting dose used was 2000 mg/kg p.o. Dose volume
was administered 0.1 ml/10 gm body weight to the rat which was fasted overnight with water *ad libitum*. Food was withheld for a further 3 to 4 hours after administration and observed for signs of toxicity. Body weight of the rats before and after termination were noted and any changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behaviour pattern were observed, and also signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

2.9 Antiulcer Activity of *Meni Lavana Chooranam* against Aspirin + Pylorus ligation induced ulceration model:

Wistar rats were divided into five groups of six in each group. Animals were placed in cages with grating floor to avoid copography in fasting period.

**Experimental protocol:**

Group I - Animals received 1% SCMC 10 ml/kg b.w (p.o).

Group II - Animals received Aspirin 200 mg/kg b.w (p.o) suspended in 1% SCMC.

Group III - Animals received *Meni lavana chooranam* 200 mg/kg b.w (p.o) suspended in 1% SCMC.

Group IV - Animals received *Menilavana chooranam* 400 mg/kg b.w (p.o) suspended in 1% SCMC.

Group V - Animals received standard drug Ranitidine 50 mg/kg b.w (p.o) suspended in 1% SCMC.

Aspirin was administered in non-fasted rats once daily for eight days for all the groups except group I animals. Ranitidine (50 mg/kg) and *Menilavana chooranam* (200,400 mg/kg) were administered orally to the respective treatment groups 30 minutes before each Aspirin treatment, whereas the control group received only 1% SCMC and fasted for 18 hrs. On the ninth day, immediately after aspirin treatment, pylorus ligation was done under ether anesthesia. Four hours after pylorus ligation, the animals were sacrificed by giving overdose of ether. The stomach was dissected out after tying the oesophageal end. The stomach was cut open along the greater curvature and the contents drained into a small beaker, centrifuged, then subjected to analysis for the following acid secretary evaluation.
2.10 Determination of gastric volume:

After sacrificing the rats, the stomach portion was removed, the gastric contents transferred into a centrifuge tube and centrifuged at 2000 rpm for 5 minutes. The supernatant liquid was transferred to a measuring cylinder and volume was measured.

2.11 Determination of gastric pH:

1ml of the gastric juice was collected and pH was directly measured by using pH meter.

2.12 Estimation of free acidity and total acidity:

1 ml of gastric juice was pipetted out into a 100 ml conical flask. 2 to 3 drops of Topfer’s reagent was added and titrated with 0.01N NaOH (which was previously standardized with 0.01N of oxalic acid) until all traces of the red color disappears and the color of solution was yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity, then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. This volume corresponds to total acidity.

Acidity was calculated by using the formula,

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual normality of NaOH}}{0.1} \times 100 \text{ mEq/l/100g}
\]

2.13 Ulcer Index:

After sacrificing the rats, the stomach was removed and opened along the greater curvature and the severity of hemorrhagic erosions in the acid secreting glandular mucosa was observed under 10 x magnifications for ulcers.

The ulcer score was assessed on a scale of 0 to 3.

0 - normal colored stomach

0.5 - red colouration

1 - Spot ulcers
1.5 - hemorrhagic streaks
2. - hemorrhagic streaks up to 4mm
3. - erosions longer than 5mm or confluent hemorrhages.

2.14 Histopathology:

The stomach sections were washed thoroughly with saline, dehydrated in ethanol and finally stained using hematoxylin and eosin. The stained sections were examined under light microscope (magnification 100x).

2.15 Statistical Analysis:

The statistical analysis of various studies was carried out using student ‘t’ test and analysis of variance (ANOVA) followed by Dunnett’s ‘t’ test, P<0.05 were considered as significant.

3. RESULTS

3.1 Acute oral Toxicity:

The test drug Meni Lavana Chooranam did not exhibit any significant toxicity at 2000 mg/kg bodyweight. So the drug is considered to be safe for long-term administration.

3.2 Antiulcer activity of Menilavana chooranam:

Table-1: Anti-ulcer activity of Meni Lavana Chooranam against Aspirin + Pylorus ligation induced ulcer in rats:

<table>
<thead>
<tr>
<th>Groupe</th>
<th>Gastric Volume (ml/100g)</th>
<th>pH</th>
<th>Free acidity (mEq/l/100gm)</th>
<th>Total acidity (mEq/l/100gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.03 ± 0.54</td>
<td>3.13 ± 0.63</td>
<td>17.06 ± 1.74</td>
<td>50.33 ± 5.85</td>
</tr>
<tr>
<td>II</td>
<td>5.16 ± 0.41a**</td>
<td>1.57 ± 0.10a**</td>
<td>39.83±7.08a**</td>
<td>84.33 ± 19.88a**</td>
</tr>
<tr>
<td>III</td>
<td>3.55 ± 0.56 b**</td>
<td>2.72 ± 0.38b*</td>
<td>23.98±3.99b**</td>
<td>59.9 ± 7.89 b**</td>
</tr>
<tr>
<td>IV</td>
<td>3.01 ± 0.12 b**</td>
<td>3.53 ± 0.28b**</td>
<td>18.45±0.71b**</td>
<td>58.63 ± 2.41 b**</td>
</tr>
<tr>
<td>V</td>
<td>3.06 ± 0.49 **</td>
<td>3.5 ± 0.71b**</td>
<td>20.82±6.17b**</td>
<td>59.2 ± 4.67 b**</td>
</tr>
</tbody>
</table>
Values are mean ± S.D of six animals in each group. Comparisons were made

a - Group I Vs Group II treated group

b - Group II Vs Group III, IV, V treated groups

* - P < 0.01

** - P < 0.001

**Gastric Volume:** The gastric volume of the Aspirin-treated animals significantly increased (P<0.001) when compared with normal control animals. *Menilavana chooranam* treated animals (200,400 mg/kg, p.o) significantly (P<0.001) reduced the gastric volume compared with Aspirin-treated animals. (Table-1).

**Gastric pH:** The pH level was significantly increased (P<0.001) in Aspirin-treated animals, when compared with control group. Administration of the *Men lava chooranam* (200 and 400 mg/kg) and standard treated animals showed significant (P<0.001) decrease in pH level when compared to Aspirin-treated animals. (Table-1).

**Free Acidity:** The free acidity of the Aspirin-treated animals significantly (P<0.001) increased, when compared with normal animals. *Men lava chooranam* (200 and 400 mg/kg) and standard Ranitidine-treated animals showed significant (P<0.001) reduction when compared with Aspirin-treated animals. (Table-1).

**Total Acidity:** The total acidity of the Aspirin-treated animals showed significant (P<0.001) increase when compared with control animals. Whereas the *Men lava chooranam* (200 and 400 mg/kg) and standard treated animals showed significant (P<0.001) decrease when compared with Aspirin-treated animals. (Table-1).
FIG: 3 Graphs showing effect of *Meni Lavana Chooranam* against Aspirin + Pylorus ligation induced ulcer in rats:

Fig: 3-A Effect of Menilavana chooranam on gastric volume Fig: 3-B Effect of Menilavana chooranam on $pH$ against Aspirin + Pylorus ligation induced ulcer in rats against Aspirin + Pylorus ligation induced ulcer in rats

Fig: 3-C Effect of Menilavana chooranam on Free acidity Fig:3-D Effect of Menilavana chooranam on Total acidity against Aspirin + Pylorus ligation induced ulcer in rats against Aspirin + Pylorus ligation induced ulcer in rats
**Ulcer Index: Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Gastric erosion scale</th>
<th>Mean ± SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>0</td>
<td>0</td>
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<tr>
<td>III</td>
<td>6</td>
<td>2</td>
<td>1</td>
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<tr>
<td>IV</td>
<td>6</td>
<td>3</td>
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<td>V</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
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<td></td>
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</tr>
</tbody>
</table>

One-way ANOVA F = 9.79 df = 4.25

0 - Normal colored stomach

0.5 - red colouration

1 - Spot ulcers

1.5 - Hemorrhagic streaks

2 - Haemorrhagic streaks up to 4 mm

3 - Erosions longer than 5 mm or confluent hemorrhages.

The gastric erosion of the Aspirin-treated animals significantly (P<0.001) increased, when compared with normal control animals, whereas the drug treated animals (200 and 400 mg/kg, p.o) significantly (P<0.01, P<0.001) reduced the gastric erosions compared with Aspirin-treated animals. The standard drug Ranitidine-treated animals also exhibited significant (P<0.001) reduction of gastric erosion (Table-2).
FIG: 4 Stomach section showing effect of *Menilavana chooranam* against Aspirin + Pylorus ligation induced ulcer in rats

*Fig: 4-A Group 1* Animals received 1% SCMC 10 ml/kg b.w  
*Fig: 4-B Group 2* Animals received Aspirin 200 mg/kg

*Fig: 4-C Group 3* Received *Menilavana chooranam* 200 mg/kg b.w  
*Fig: 4-D Group 4* Received *Menilavana chooranam* 400 mg/kg

*Fig: 4-E Group* standard drug Ranitidine 50 mg/kg  
*Fig: 4-F ulcer index*

*Citation: Hemalatha P et al. Ijppr.Human, 2016; Vol. 7 (4): 294-309.*
FIG: 5 Histopathology

Fig: 5-A Group 1

Fig: 5-B Group 2

Fig: 5-C Group 3

Fig: 4 Group 4

Fig: 5 Group 5
Group I: Section shows normal gastric mucosa with no ulceration.

Group II: Section shows gastric mucosa with ulcer covered by exudate glands appear hyperplastic.

Group III: Shows hyperplastic gastric mucosa with intact epithelial layering submucosal edema is seen.

Group IV: Shows normal gastric mucosa, no ulcer, congested vessels in the submucosa with mild edema

Group V: Shows gastric mucosa with focal superficial erosion.

DISCUSSION

Immorality in the dietary habits results in Gastro-Intestinal disorders. According to Siddha system, gastrium i.e., stomach is the main seat for several diseases. So there arises a need to take some steps for regulating and treating Gastro Intestinal disorders. The proof of pharmacological activities that are available at present is mostly based on empirical experience. The main problem facing the traditional medicine is proof requirement that the active principles contained in medicinal plants are useful, safe and effective. Hence a study on Menilavana chooranam to evaluate its antiulcer activity was done based on the evidence collected from the Siddha literature. Menilavana chooranam is a herbomineral product made with easily available ingredients. Literature evidence shows that all the ingredients of Menilavana chooranam play significant role in treatment of peptic ulcer. In Siddha gunman disease symptoms are similar to peptic ulcer symptoms. In gunman vatham gets deranged. The trial drug Meni Lavana chooranam has predominantly acrid and salt taste. Both these tastes, chiefly compromise Thee (fire) bootham which pacifies the deranged vatham. Thus the Menilavana Chooranam acts as ethirurai medicine in the treatment of gunman as per Siddha concept.

Acute toxicity of Menilavana chooranam was studied and the drug was proved safe for long-term administration as it did not exhibit any significant toxicity at 2000 mg/ kg body weight. Antiulcer activity of Menilavana Chooranam gave significant results. Menilavana chooranam treated animals (200, 400 mg/kg, p.o) significantly (P<0.001) reduced the gastric volume compared with Aspirin-treated animals. (Table-1). Administration of the Meni lavana chooranam (200 and 400 mg/kg) and standard treated animals showed significant (P<0.001)
decrease in pH level when compared to Aspirin-treated animals. (Table-1). *Menilavana chooranam* (200 and 400 mg/kg) and standard Ranitidine-treated animals showed significant (P<0.001) reduction when compared with Aspirin-treated animals. (Table-1). The total acidity of the Aspirin-treated animals showed significant (P<0.001) increase when compared with control animals. Whereas the *Menilavana chooranam* (200 and 400 mg/kg) and standard treated animals showed significant (P<0.001) decrease, when compared with Aspirin-treated animals. (Table-1). *Menilavana chooranam* treated animals (200 and 400 mg/kg, p.o) significantly (P<0.01, P<0.001) reduced the gastric erosions compared with Aspirin-treated animals. The standard drug Ranitidine-treated animals also exhibited significant (P<0.001) reduction of gastric erosion (Table-2). The antiulcer activity may be due to presence of active substances such as flavonoids in the trial drug. The results of the pharmacological study give scientific evidence to prove the efficacy of the *Menilvana Chooranam* in the treatment of peptic ulcer. *Menilavana chooranamis* found to be effective pharmacologically.

**CONCLUSION**

The present study indicates that the Siddha herbomineral formulation *Menilvana choornam* has potential antiulcer activity against aspirin plus pylorus ligation induced ulcer in rats. The experimental findings provide scientific support to the traditional use of *Menilvana chooranam* in the treatment of peptic ulcer.

**CONFLICT OF INTEREST**

Authors declare no conflict of interest.

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