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Anti-secretory and anti-ulcer activity of Surasa, A poly herbal formulation in pylorus ligated (Shay) rat model

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ABSTRACT

Surasa is herbal formulation consisting of *Phyllanthus embelica, Withania somnifera, Glycyrrhiza glabra, Cuminum cyminum, Eclipta alba* and Shanka bhasma. In the present study anti-secretory and ulcer protective effects of Surasa was evaluated in pylorus-ligated rats' model. Wistar rats weighing 180-200g were divided into seven groups of six animals each and were fasted for 36 hours before pylorus ligation, with water provided *ad libitum*. Ranitidine (50mg/kg.b.w) was administered to standard group. Test groups received Surasa at 3 dose levels: 137, 205, 274mg/kg.b.w for acute study and at 2 dose levels of 137 and 205 mg/kg for chronic study. For acute study treatments were administered orally one hour prior to pylorus ligation.[3] For chronic study animals were treated for seven days. After the treatment, pylorus ligation was carried out under ether anesthesia. Animals were sacrificed four hours after pylorus ligation with overdose of anesthetic ether. Gastric juice was collected and volume, pH, free acidity and total acidity were measured. The stomachs were cut opened and inner surface was examined to determine ulcer index. In acute study Surasa, significantly ($p< 0.05$) reduced gastric secretions and ulcer score. Antisecrtroy and antiulcer activity of Surasa was very significant ($p<0.01$) with chronic treatment.

Key words: Herbal formulation**,** Gastric acid, gastric ulcers, Pylorus ligation.

INTRODUCTION

Gastric ulcer is a common disorder caused due to imbalance between aggressive (acid, pepsin, *H. Pylori*) and defensive (gastric mucus, bicarbonate secretion, prostaglandin) factors. A number of drugs like H2 receptor antagonists, gastric anti-secretory drugs, anti- muscarinic agents, proton pump inhibitors, mucosal protective agents are available for treatment of gastric ulcers, but are associated with side effects and limitations[1]. The major thrust area of research at present revolves around the search for the indigenous drugs which may be better and safer alternative for the treatment of gastric ulcers. Surasa is an herbal formulation consisting of *Phyllanthus embelica, Withania somnifera, Glycyrrhiza glabra, Cuminum cyminum, Eclipta alba* and Shanka bhasma. Based on the varied properties of these components, investigation of its effect on gastric acid secretion and gastric ulcers was carried out.

MATERIAL AND METHODS

The constituent of Surasa, were procured from authentic sources and identified by Dr. Shubha Hegde of Srushti herbal Pharma Bangalore. All the constituents of the formulation were weighed individually and mixed (Table 1). A voucher specimen has been deposited at the library of Srushti herbal Pharma Bangalore. Drugs were administered as oral aqueous suspensions (0.5% sodium CMC solution).

Wistar rats weighing 180-200g were obtained from Drug testing laboratory Bangalore.

Table 1: Constituents of surasa

Ingredients	Botanical name	Part used	Quantity
Amalaki	*Phyllanthus emblica*	Fruit	100mg
Ashwagandha	*Withania somnifera*	Stem and Root	80mg
Yashtimadu	*Glycyrrhiza glabra*	Dried roots	60mg
Jeeraka	*Cuminum cyminum*	Seeds	20mg
Bhringaraja	*Eclipta alba*	Stem and leaves	60mg
Shanka bhasma	Incinerated counch	Counch shell	60mg

Approval for the animal experiments was obtained from Institutional Animal Ethics Committee (IAEC) of Government College of Pharmacy, Bangalore. Animals were maintained and handled as per CPCSEA guidelines. They were fed with standard rat feed and purified water ad-libitum.

Acute oral toxicity study

Acute oral toxicity study was performed according to the OECD (Organization for Economic Co-operation and Development) guidelines 425[6]. Albino rats of Wistar strain (180-200g) were maintained under controlled standard animal house conditions with access to food and water ad libitum. The rats were acclimatized for 5 days and fasted overnight, food but not water was withheld. The limit test was carried out first at 5000mg/kg body weight for one animal and if animal died, main test was performed. If the animal survived two more animals were dosed, if both survive the test was terminated. The main test was performed with an initial dose of 175mg/kg body weight and using half log units and sequence followed was 175, 550, 1750 and 5000 mg/kg body weights.

The dosing was stopped when one of the following stopping criteria was met.

- Consecutive animals survive at the upper bound
- Reversals occur in any 6 consecutive animals tested
- At least four animals have followed the first reversal and the specified likelihood ratios exceed the critical value.

Experimental protocol

The rats were divided into 7 groups of six animals each. Animals were allowed to fast for 36 hours before pylorus ligation, with water provided *ad libitum*.[2] Care was taken to avoid coprophagy. Control group received vehicle (0.5% of sodium CMC in distilled water) only. Ranitidine (50mg/kg b.w) was administered to standard group. The test groups received Surasa at three dose levels: 137, 205 and 274mg/kg b.w for acute study and at two dose levels of 137 and 205 mg/kg for chronic study. For acute study treatments were administered orally one hour prior to pylorus ligation[3]. For chronic study animals were treated for seven days and on seventh day pylorus ligation was carried out. Doses of Surasa were extrapolated from the intended human dose.

Under light ether anesthesia, the abdomen was cut opened by a small midline incision below the xiphoid process, pyloric portion of the stomach was slightly lifted out and ligated, avoiding traction to the pylorus or damage to its blood supply.[4] Stomach was then replaced carefully and abdominal wall closed by interrupted sutures.

The animals were sacrificed four hours after pylorus ligation with overdose of anesthetic ether. Stomachs were dissected out and its contents were drained into centrifuge tubes. Gastric juice collected from the pylorus-ligated rats was centrifuged (3000 RPM for 10 min.). Volume and pH of gastric juice was measured.

Determination of free and total acidity[5]

Gastric juice collected, was taken in a 100 ml conical flask. 2-3 drops of Topfer's reagent was added and titrated to pH 3.5 with 0.1 N NaOH (which was previously standardized with 0.01N oxalic acid) until all traces of the red colour disappeared and the colour of solution was yellowish orange. The volume of alkali added was noted which corresponds to free acidity. Then 2-3 drops of phenolphthalein solution was added and titration was continued up to pH 8.0, until a definite red tinge reappears. Again the total volume of alkali added was noted which corresponds to total acidity. Acidity was calculated by using the formula:

Acidity = Volume of NaOH x Normality of NaOH x 100 / 0.1 (meq/l per 100g)

Determination of ulcer index [6]

The stomachs after the removal of its contents were cut opened along the greater curvature. The inner surface was examined for ulceration. Severity of ulcers was recorded with the following scores

0 = no ulcers
2 = deep ulcers
1 = superficial ulcers
3 = perforation

Ulcer index U_I is calculated as

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

U_N = average of no of ulcers per animal
U_S = average of severity of score (0, 1, 2, 3)
U_P = percentage of animals with ulcers

RESULTS

Statistical analysis

Data is expressed, as the mean ± SEM. Comparison among groups was statistically processed by the one-way analysis of variance (ANOVA) with Tukey's post hoc analysis in "Graph Pad InStat" software. P value less than 0.05 were considered as significant.

Acute oral toxicity study

The Ostinu is found to be safe up to a dose of 5000mg/kg body weight, as on gross observation no untoward effects were seen.

Acute study

(Table 2, 3 and 4): Surasa 137 mg/kg b.w significantly reduced gastric ulcer score when compared to control, however it had no effect on gastric volume, gastric pH, free acidity and total acidity. Surasa 205 mg /kg b.w significantly reduced gastric volume, free acidity, total acidity and gastric ulcer score compared with control. Surasa 274 mg /kg b.w and Ranitidine 50mg/kg showed very significant effect on gastric pH, gastric volume, free acidity, total acidity and gastric ulcer score compared with control.

Chronic Study

(Table 5, 6 and 7): Surasa 137 mg /kg b.w has significant effect on gastric pH, free acidity, total acidity, ulcer score and very significant effect on gastric volume.

Table 2: Acute study, effect of Surasa on gastric volume and pH

Group	Gastric Volume(ml)	Gastric pH
Control	6.66 7±0.247	1.455 ± 0.138
Surasa 1 (137mg)	5.833 ± 0.333	1.923 ± 0.149
Surasa 2 (205mg)	5.500± 0.182*	2.058 ± 0.255
Surasa 3 (274mg)	3.833±0.278**	2.967± 0.309*
Ranitidine (50mg)	2.333±0.247 **	3.658±0.598**

Table 3: Acute study, effect of surasa on free and total acidity

Group	Free Acidity(mg eq /l per 100g)	Total Acidity(mg eq / l per 100g)
Control	483.33 ± 42.16	643.33 ± 42.32
Surasa 1 (137mg)	380.00 ± 55.01	545.00 ± 51.86
Surasa 2 (205mg)	293.33 ± 30.405*	455.00 ± 25.66*
Surasa 3 (274mg)	265.00 ± 60.48**	445.00 ± 62.70*
Ranitidine (50mg)	106.67 ± 35.93**	263.33 ± 45.22**

Table 4: Acute study, effect of surasa on ulcer score and ulcer index

Group	Ulcer Score	Ulcer Index
Control	2.50 ± 0.341	16.16
Surasa 1 (137mg)	1.33 ± 0.333*	11.99
Surasa 2 (205mg)	1.33 ± 0.333*	11.49
Surasa 3 (274mg)	0.83 ± 0.307**	8.499
Ranitidine (50mg)	1.00 ± 0.258**	10.49

Table 5: Chronic study, effect of surasa on gastric volume and pH

Group	Gastric Volume(ml)	Gastric pH
Control	6.66 7 ± 0.247	1.455 ± 0.138
Surasa 1 (137mg)	4.250 ± 0.495**	3.057 ± 0.303*
Surasa 2 (205mg)	3.000 ± 0.695**	3.705 ± 0.588**
Ranitidine (50mg)	2.333 ± 0.247 **	3.658 ± 0.598**

Table 6: Chronic study, effect of surasa on free and total acidity

Group	Free Acidity(mg eq /l per 100g)	Total Acidity(mg eq / l per 100g)
Control	483.33 ± 42.16	643.33 ± 42.32
Surasa 1 (137mg)	266.67 ± 57.716*	421.67 ± 53.255*
Surasa 2 (205mg)	160.00 ± 72.065**	333.33 ± 61.028**
Ranitidine (50mg)	106.67 ± 35.93**	263.33 ± 45.22**

Table 7: Chronic study, effect of surasa on ulcer score and ulcer index

Group	Ulcer Score	Ulcer Index
Control	2.50 ± 0.341	16.16
Surasa 1 (137mg)	1.33 ± 0.210*	10.49
Surasa 2 (205mg)	0.833 ± 0.307**	8.49
Ranitidine (50mg)	1.00 ± 0.258**	10.49

Surasa 205 mg /kg b.w and Rantidine 50mg/kg b. w used as standard, has very significant effect on gastric volume, gastric pH, free acidity, total acidity and gastric ulcer score.

DISCUSSION

In the present study antisecretory and ulcer protective effects of Surasa was evaluated in pylorus-ligated rats' model. Gastric ulcers have multiple etiopathogenesis. In pylorus ligated rats ulcers are caused by increased presence of acid and pepsin in the stomach[8]. Gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats[4]. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hypersecretion model of pylorus ligature is believed to increase gastric acid secretion.[9]. The present study clearly demonstrated that, Surasa dose-dependently decreased gastric acid secretion. Saponins, tannins and volatile oil of some plants are known to possess antiulcer activity.[11] In the present study active principles responsible for the anti-ulcer activity were not studied.

In conclusion, Surasa has showed significant anti-secretory and anti ulcer activity in pyloric ligated (Shay) rat model. The activity is dependent on dose and duration of treatment. Further experiments and detailed phytochemical analysis are required to determine the phytoconstituent(s) responsible for anti-secrotory and anti-ulcer mechanisms involved.

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