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AN *IN-VIVO* STUDY TO EVALUATE THE ANTI-ULCER ACTIVITY OF *NĀRIKELĀ LAVANA* IN PYLORIC LIGATION INDUCED ULCER IN WISTAR ALBINO RATS

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Keywords:

Narikela lavana, Antiulcer study, *In-vivo* anti-ulcer study

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ABSTRACT: *Lavana kalpanas* are pharmaceutical preparation in which the '*Lavana*' and the selected '*auṣadhadravyā'* are igniting together in a closed *sampuṭā* to obtain the drug ash as medicinal product. *Nārikelā lavana*, one among the *lavana kalpana* mentioned in *Bhaiṣajyaratnāvali* 30th chapter and in *Rasa taraṇgiṇi*14th chapter. *Nārikelā lavaṇa* proved to have a high amount of electrolytes like calcium and potassium, which have antacid properties. Here the common ingredient in this *Kalpana* is *lavana*, which possesses *madhura-lavanarasa* & *śītaviryā* and having *pittahara* property which might be the reason for the extensive use of *Lavaṇa Kalpanas*in *pittajanyavikaras*. The higher alkalinity of *Nārikelā lavaṇa* may also play an important role in its mode of action. **Materials and Methods:** *Nārikelā lavana* was prepared as per the reference in *Rasa taraṇgiṇi*, and analytical parameters were tested. The experimental study was conducted in pyloric ligated Wistar albino rats. Then the data were analyzed statistically using One Way ANOVA followed by Dunnett's multiple "t" test as a post hoc test. **Results & Discussion:** The analytical parameters like pH, LOD, Total ash, Acid insoluble ash, water-soluble ash etc. were tested, and the results were found to be within the permeable limits. The trial drug (Nārikelā lavana) shows significant results in total acidity, free acidity, and ulcer index by analyzing various parameters indicating the anti-ulcer activity. **Conclusion:** The obtained results clearly indicate *Nārikelā lavana* possesses anti-ulcer activity.

INTRODUCTION: Peptic ulcer is the most predominant gastrointestinal disease; current therapy for Peptic ulcer is H2-receptor blockers, proton pump inhibitors, antacids, anticholinergics, and antibiotics. Currently available treatments have limited efficacy and severe side effects. Hence, ulcer treatment is one of the challenging problems, and the researchers are looking forward to a drug with the minimal side effect, easily accessible & affordable.

Nārikelā lavaṇa, one among the *Lavana kalpana* mentioned in *Bhaiṣajyaratnāvali* 30th chapter and in *Rasa taraṇgiṇi* 14th chapter. Its main indication mentioned in both texts are *śūla*, especially in *pariṇāmaśūla*. By analyzing symptoms *śūla*c an be co-related to peptic ulcers.

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Nārikelā lavana was proved to have a high amount of electrolytes like calcium and potassium, which has antacid properties. Here the common ingredient in this *Kalpana* is *lavana*, which possess *madhura-lavanarasa* & *śītaviryā* and having *pittahara* property which might be the reason for the extensive use of *Lavaṇa kalpanas* in *Pittajanyavikaras*. The higher alkalinity of *Nārikelā lavaṇa* may also play an important role in its mode of action.

Aims and Objectives: To analyze the pysico-chemical properties of *Nārikelā lavaṇa* and to *experimentally* evaluate the anti-ulcer activity of *Nārikelā lavaṇa*.

MATERIALS AND METHODS

Pharmaceutical Study: Pharmaceutically authentic and pure drugs were collected, and later *Nārikelā lavaṇa* was prepared according to the reference from *Rasa taraṇgiṇi* [1]. *Nārikelā lavaṇa* consists of only 2 ingredients- *nārikelā* and *Saindhavā lavaṇa*. A mature coconut devoid of outer fibrous part is taken & a hole is made into one of its eyes, and all the liquid inside is poured out. *Saindhavā lavaṇa* should be powdered well and poured into the coconut through the hole. The hole is now plugged with a mud cork. A layer of *multanimitt*i smeared cloth was covered on the coconut and allowed it to dry, and the process was repeated 3 times. After proper drying of layers, it was subjected to *Mahaputa* [2]. The temperature was taken every 10 min using a thermocouple.

Analytical Study: *Nārikelā lavaṇa* was analyzed by doing the following tests: -Loss on Drying (LOD), Total ash, Acid insoluble ash, Water soluble ash, pH (5% solution) [3, 4].

Experimental Study: Wistar strain albino rats weighing between 160 to 250g of either sex were used for the study. The Institutional Animal Ethical Committee approved all experimental protocols following the guideline formulated by CPCSEA and Approval No. SDMCRA/IAEC/AM-R-01. In pyloric ligation-induced ulcers, an experimental study was conducted in 3 groups, *i.e.*, control, standard and trial groups with 8 Wistar albino rats, which were randomly selected. A day before dosing, the selected animals would be randomly divided into three groups comprising 4 male and 4 female Wistar albino rats.

The test drugs would be administered for 7 consecutive days. Ranitidine was administered for 4 consecutive days prior to pyloric ligation for the reference standard group. The control group, CMC solution and distilled water were also given for 7 consecutive days. Animals would be fasted for 36-40 hours by placing them in metabolic cages to prevent coprophagy but provided free access to water ad libitum. The dosing would be continued during this period. On the tenth day, one hour after dosing, pylorus was ligated by the method of Shay *et al.* (1945) [5].

Pre Operative: Rats were anesthetized with Inj. Ketamine 80mg/kg body weight (Intraperitoneal) **Fig. 1** and Inj. Xyloxine 3mg/kg body weight (Intra Muscular) **Fig. 2** for analgesic action. Shaving of the ventral part of the abdomen of rats was done [6] **Fig. 3.**

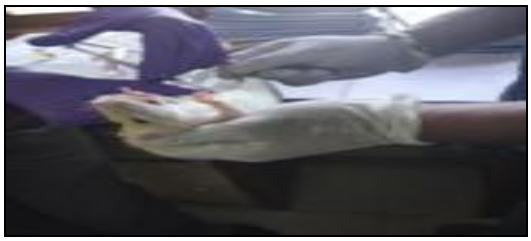

FIG. 1: FIG. 1: KETAMINE INJECTION (IP)

FIG. 2: XYLOXINE INJECTION (IM)

FIG. 3: SHAVING OF VENTRAL PART

Operative Procedure: On the ventral part of the abdomen portion was opened in a layer by a small midline incision just below and lateral to the xiphoid process **Fig. 4**. Pyloric portion of the stomach was slightly lifted out, avoiding traction to the pylorus or damage to its blood supply. The pylorus was ligated with linen thread No.10 and stomach was replaced carefully **Fig. 5**. The incision was closed with interrupted sutures in layers & betadine ointment was applied over the sutures [7].

FIG. 4: ANESTHETIZED RAT ON DISSECTION TABLE

FIG. 5: PYLORIC LIGATION

Post Operative Procedure: Each rat was kept in an individual metabolic cage. The animals were deprived of both food and water. Animals were euthanized under deep ether anesthesia at the end of 10 h after pyloric ligation [8]. The abdominal cavity was reopened carefully, and the stomach was excised after tying the esophageal end to prevent loss of gastric contents during excision **Fig. 6**. Gastric contents were drained into tubes and centrifuged at 2000 rpm for 10 min **Fig. 7**. The gastric juice's volume and pH were noted and used for biochemical estimation. The stomach was opened along the greater curvature and washed under the running tap water. Then it was fixed on a wax board & observed the ulcers using a magnifying lens **Fig. 8**. After assessing the ulcer score, the glandular portion of the stomach was sent for histopathological assessment [9, 10].

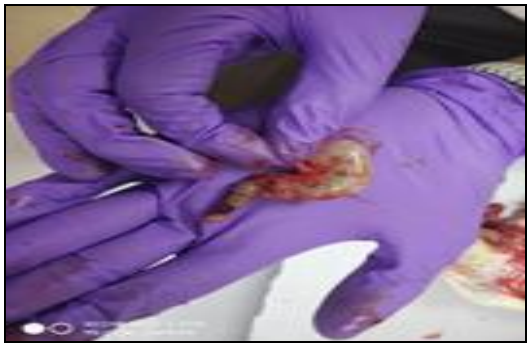

FIG. 6: PYLORIC LIGATED STOMACH

FIG. 7: COLLECTION OF GASTRIC JUICE

FIG. 8: ULCER ASSESSMENT

RESULTS: The data obtained were analyzed using Graph pad in stat version 3.05 by student "t" test for comparison between two positive control groups and the rest of the data were analyzed by One Way ANOVA followed by Dunnett's multiple "t" test as a post hoc test for determining the level

of significance of the observed effects. Eight parameters were analyzed during the study.

pH of Gastric Juice: The data in the **Table 1** shows there was an increase in gastric pH in the standard group when compared to the control group, the observed increase was found to be statistically very significant and there was an increase in gastric pH in test group when compared to the control group, the observed increase was found to be statistically non-significant.

TABLE 1: EFFECT OF *NĀRIKELĀ LAVANA* ON PH OF GASTRIC JUICE

Group	pH	% change
Control	2.71±0.28	
Standard	5.75±0.25**	112.17↑
Test	3.58±0.61	32.10↑

Data: MEAN±SEM, **p<0.01.

Volume of Gastric Juice: The data in the **Table 2** shows there was a decrease in gastric juice volume in the standard group when compared to the control group, the observed decrease was found to be statistically non-significant and there was an increase in gastric juice volume in the trial group when compared to the control group, the observed increase was found to be statistically non-significant.

TABLE 2 EFFECT OF *NĀRIKELĀ LAVANA* ON VOLUME OF GASTRIC JUICE

Group	Volume	% change
Control	1.57±0.46	
Standard	0.75±0.18	52.22↓
Test	2.75±0.65	75.15↑

Data: MEAN±SEM.

Free Acidity: The data in the **Table 3** shows there was a decrease in free acidity in the standard group when compared to the control group, the observed decrease was found to be statistically significant, and there was a decrease in free acidity in the test group when compared to the control group, the observed decrease was found to be statistically very significant.

TABLE 3 EFFECT OF *NĀRIKELĀ LAVANA* ON FREE ACIDITY

Group	Free acidity	% change
Control	2.86±0.19	
Standard	1.9±0.00*	33.56↓
Test	1.26±0.10**	55.94↓

Data: MEAN±SEM, *P<0.05, **P<0.01

Total Acidity: The data in the **Table 4** shows there was an increase in total acidity in the standard group when compared to the control group, the observed increase was found to be statistically non-significant and there was a decrease in total acidity in the test group when compared to the control group, the observed decrease was found to be statistically significant.

TABLE 4 EFFECT OF *NĀRIKELĀ LAVANA* ON TOTAL ACIDITY

Group	Total acidity	% change
Control	5.42±0.31	
Standard	6±0.00	10.70↑
Test	4.32±0.27*	20.29↓

Data: MEAN±SEM, *P<0.05.

Ulcer Index: The data in the **Table 5** shows there was a decrease in ulcer index in the standard group, when compared to the control group, the observed decrease was found to be statistically very significant, and there was a decrease in ulcer index in the test group when compared to the control group, the observed decrease was found to be statistically very significant.

TABLE 5: EFFECT OF *NĀRIKELĀ LAVANA* ON ULCER INDEX

Group	Ulcer index	% change
Control	10.85±2.25	
Standard	3±0.82**	72.35↓
Test	4±0.87**	63.13↓

Data: MEAN±SEM, **P<0.01

Carbohydrate Estimation: The data in the **Table 6** shows there was a decrease of total carbohydrates in standard group when compared to the control group, the observed decrease was found to be statistically very significant and there was decrease in total carbohydrate in test group when compared to the control group, the observed decrease was found to be statistically non-significant.

TABLE 6: EFFECT OF *NĀRIKELĀ LAVANA* ON CARBOHYDRATE ESTIMATION

Group	Total carbohydrate	% change
Control	1809.2±232.65	
Standard	706.2±143.90	60.96↓
Test	1193.2±169.08	34.04↓

Data: MEAN±SEM, **P<0.01

Protein Estimation: The data in the **Table 7** shows there was a decrease in total protein in the standard group when compared to the control group, the observed decrease was found to be

statistically non-significant, and there was decrease in total protein in test group when compared to the control group, the observed decrease was found to be statistically non-significant.

TABLE 7: EFFECT OF *NĀRIKELĀ LAVANA* ON PROTEIN ESTIMATION

Group	Total protein	% change
Control	15788±1956.0	
Standard	10141.2±1460.2	564680↓
Test	12720±2761.6	19.43↓

Data: MEAN±SEM

Peptic Activity: The data in the **Table 8** shows there was increase of peptic activity in the standard group when compared to the control group, the observed increase was found to be statistically non-significant and there was a decrease in peptic activity in test group when compared to the control group, the observed decrease was found to be statistically non-significant.

TABLE 8: EFFECT OF *NĀRIKELĀ LAVANA* ON PEPTIC ACTIVITY

Group	Peptic activity	% change
Control	360±64.797	
Standard	537.8±98.090	49.38↑
Test	345.6±87.782	4↓

Data: MEAN±SEM

Histopathological Examination of Stomach Tissue: Histopathological examination of stomach tissues was done and the following results were obtained.

Control Group: Stomach tissue sections of normal control group rats showed normal cytoarchitecture. **Fig. 9 & 10.** The data in **Tables 9, 10,** and **11** show the results of histopathological examination of stomach tissue of the control group, standard group, and trial group, respectively.

TABLE 9: SHOWING THE RESULT OF HISTOPATHOLOGY OF THE CONTROL GROUP

Group and rat no	Mucosal layer	Sub-mucosal layer	Muscular layer	Remarks
C1A3	Small area ulcerated, extending from mucosa to submucosa. Inflammatory cells seen. Loss of glandular architecture seen	No changes	No changes	Ulceration and inflammation
C2B2	Small area shows damaged epithelium	Edematous and inflamed Fig. 11	Inflammatory cells seen	Inflammation
C2B3	Small eroded area seen. Glandular architecture lost in some areas	Edema and inflammation	No changes	Erosion. Fig. 12 severe inflammation

Standard Group

TABLE 10: SHOWING THE RESULTS OF HISTOPATHOLOGY OF STANDARD GROUP

Group and rat no	Mucosal layer	Sub-mucosal layer	Muscular layer	Remarks
S1A1	No necrosis or erosion, Glandular architecture maintained. Fig. 13, 14	Inflammatory cells	No changes	Inflammation
S1A4	No necrosis or erosion, Glandular architecture maintained	Inflammatory cells	No changes	Inflammation
S2A2	No necrosis or erosion, Glandular architecture maintained	Inflammatory cells	No changes	Inflammation

Trial Group

TABLE 11: SHOWING THE RESULTS OF HISTOPATHOLOGY OF THE TRIAL GROUP

Group and rat no	Mucosal layer	Sub-mucosal layer	Muscular layer	Remarks
G1A1	No necrosis or erosion, Fig. 15 Glandular architecture changed in one area. Inflammatory cells like eosinophils, lymphocytes seen.	Inflammatory cells	No changes	Inflammation
G1A2	Glandular architecture maintained. No necrosis or erosion	Inflammatory cells	Inflammatory cells	No ulcer, erosion, necrosis. Fig. 16 Inflammation
G1A3	Glandular architecture maintained. No necrosis or erosion. Many Inflammatory cells like eosinophils, lymphocytes seen	Inflammatory cells	Inflammatory cells	No necrosis, ulcer, erosion. Inflammation seen

Photomicrograph of Histopathology of Normal Stomach Tissue:

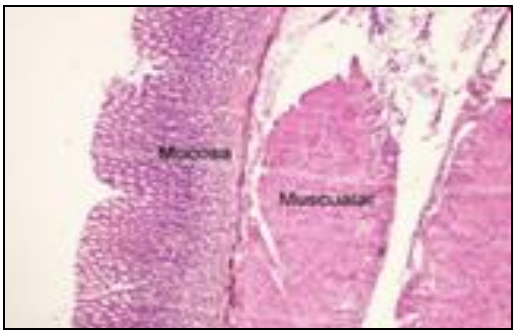

FIG. 9: NORMAL STOMACH TISSUE

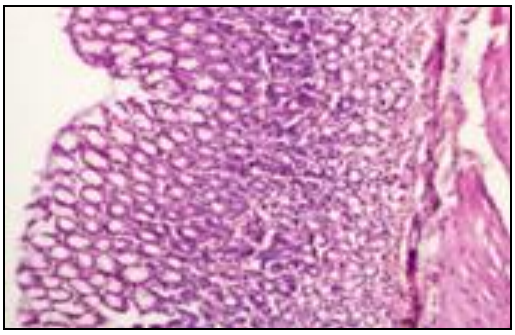

FIG. 10: ABSENCE OF NECROSIS/EROSION

Photomicrograph of Histopathology of Control Group:

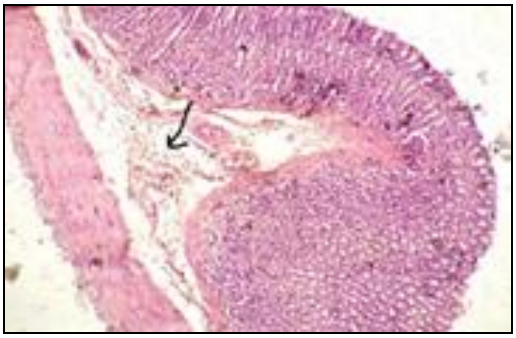

FIG. 11: ODEMA AND INFLAMMATION OF SUBMUCOSA

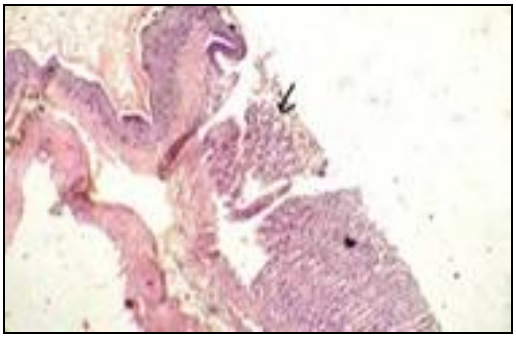

FIG. 12: MUCOSAL EROSION

Photomicrograph of Histopathology of Standard Group:

ABSENCE OF ULCER /EROSION

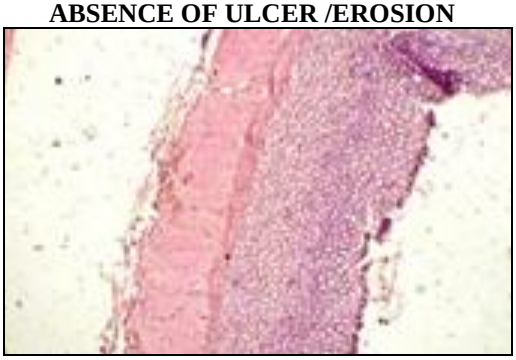

FIG. 13: ABSENCE OF ULCER

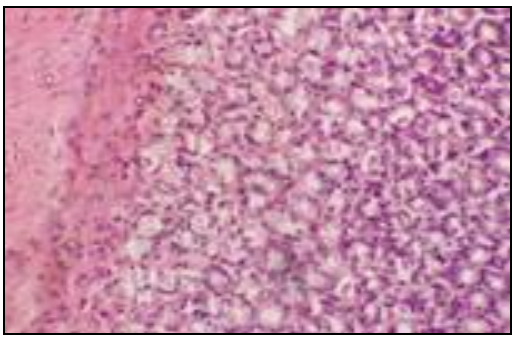

FIG. 14: MUCOSAL EROSION ABSENT

Photomicrograph of Histopathology of Trial Group:

ABSENCE OF ULCER / EROSION

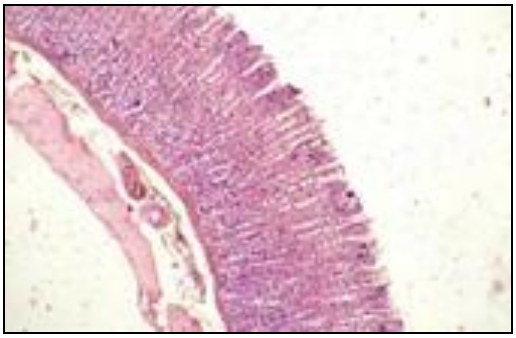

FIG. 15: ULCERATED TISSUES NOT SEEN

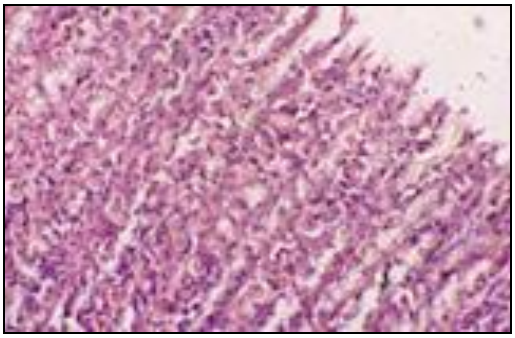

FIG. 16: ABSENCE OF MUCOSAL EROSION

DISCUSSION: The outcome of the experimental study has been provided in the form of a consolidated table as follows for easy comparison and discussion. Data in **Table 12** shows the SD in total acidity, free acidity & ulcer index, indicating the anti-ulcer activity. A decrease in the total acidity implies reduced HCl along with organic acids. A decrease in the free acidity implies reduced HCl secretion. A decrease in ulcer index implies prevention of ulcer formation. By Analysing the ayurvedic pharmacological properties of the formulation, the main ingredients - *Nārikelā* & *Saindhavā lavaṇa* possess *Madhural avanarasa*, *śītavīrya*, *laghu-snigdhaguna*, *madhuravipāka* and *vātapittahara* properties. *Madhura* and *lavanarasa* of the formulation will pacify the vitiated *vāta*. *Madhura vipāka* and *śītavīrya* of the formulation may increase the mucosal secretion and thereby preventing the ulceration. *Madura rasa* is *Sandhanakrit,* which also might help in healing the ulcer. Pharmacological studies on the drug, *Nārikelā* revealed the presence of alkaloids, tannins, resins, and phenolic compounds such as terpenoids, steroids *etc* [11].

Tannin and alkaloids were proved to be having anti-ulcer activity, which prevents ulceration [12]. The presence of phenolic compounds acts as anti-oxidant, that reduces the formation of free radicals and thus potentially protects the cell from oxidative damage [13]. An analytical study on the formulation revealed higher alkalinity of 9.64, shows the significant effect on preventing the ulceration. Ash value was found to be 90.84%, indicating the richness of minerals present in the sample, which inturn raises its therapeutic efficiency. Higher water-soluble ash (87.4%) and lower acid insoluble ash (1.24%) in turn indicating its higher solubility and the absence of impurities, respectively.

Low LOD value (1.04%) ensures a longer shelf life of the sample. In an *in-vivo* study, a significant decrease in total acidity, free acidity and ulcer index were found compared to the pyloric ligated control group. Phytochemicals like tannin, and alkaloids were proved to have anti-ulcer activity by reducing the acid secretion might be the reason behind this significant decrease. The presence of phenolic compounds acts as anti-oxidant, thus repairing the damaged cells, and higher alkalinity of the formulation also prevents the formation of an ulcer. In the histopathology of the test drug group, the ulceration was found to be minimal when compared to control group. While considering the overall factors related to the mechanism of healing ulcer, the main three things to be considered are acid neutralization, reducing the secretion and mucosal protection.

Saindhavā lavaṇa in the formulation helps in neutralization and reducing the acid secretion. *Nārikelā*, in the formulation proved to have anti-oxidant might help in mucosal protection. By considering these factors, we can claim that our formulation *Nārikelā lavana* act in preventive way than curative in ulceration.

TABLE 12: SHOWING THE RESULTS OF VARIOUS PARAMETERS TESTED IN GASTRIC JUICE OF WISTAR ALBINO RATS

Parameters	Standard	Trial
pH	SI	NSI
Volume of gastric juice	NSD	NSI
Total acidity	NSI	SD
Free acidity	SD	SD
Ulcer index	SD	SD
Carbohydrate estimation	SD	NSD
Protein estimation	NSD	NSD
Peptic activity	NSI	NSD

Where, NSD- Non significant decrease; SD- significant decrease; NSI- Non significant increase; SI-Significant increase

CONCLUSION: The analytical parameters like pH, LOD, Total ash, Acid insoluble ash, Water soluble ash etc were tested and the results was found to be within the permeable limits. The trial drug (*Nārikelā lavana*) shows significant results in total acidity, free acidity, and ulcer index by analyzing various parameters indicating the anti-ulcer activity. Thus, the obtained results clearly indicate *Nārikelā lavana* possesses anti-ulcer activity.

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CONFLICTS OF INTEREST: We declare no conflicts of interest

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