

## Anti ulcer activity of *Narikela Khanda* & *Narikela Khanda granules*

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**ABSTRACT :** Today in the modern era, gastric disorders like hyperacidity and gastric ulcers are a common clinical entity. This is also a major health problem with multifunctional etiology. Today there is need for a potent herbal formulation which can cure hyperacidity and ulcers. The present comparative experimental study was planned to see the anti ulcer activity of herbal compound formulations of *Narikela Khanda* and *Narikela Khanda granules* using pyloric ligation induced gastric ulceration in rats. Gastric ulcers were induced in albino rats by pyloric ligation. Effects of both the test formulations on different parameters in gastric juice like volume, pH, total and free acids, peptic activity, total carbohydrate (TC), total protein (TP) and also gastric mucosal damage were assessed. Both the formulations did not produce significant effect on gastric juice volume, free acidity, total protein, total carbohydrate, TC:TP ratio and ulcer index, however *Narikela Khanda granules* produced significant increase of gastric juice pH and significant decrease in total acidity and peptic activity. The study clearly indicates that *Narikela Khanda* possesses weak anti-ulcer activity, while *Narikela Khanda granules* possess moderate anti-ulcer activity against pyloric ligation induced gastric ulcers.

**Key words :** *Narikela Khanda*, Granules, *Amlapitta*, Peptic ulcer, Pyloric ligation, Anti ulcer activity.

### INTRODUCTION

Gastric disorders like hyperacidity and ulcers are a common clinical entity. These are major health problem with multifunctional etiology. The development of gastric ulcers occurs with acid and the breakdown of mucosal defense. Local mechanisms implicated in mucosal defense are mucous like alkaline secretions, mucosal hydrophilic activity, rapid epithelial cell renewal, rich mucosal blood flow and increased resistance of gland cells in deep mucosa to acid and peptic activity.<sup>1</sup>

A rational therapy for gastric ulcers still remains elusive and search for safer potential drugs is being carried out. Use of natural drugs in gastric ulcers is well documented. Most of these drugs augment the mucosal defensive factors, which are thought to be important for protection of gastric mucosa.<sup>2,3</sup>

*Narikela (Cocos nucifera* Linn.) is commonly available fruit which is consumed almost daily in Indian food and its use in the medicine is also not uncommon. Many preparations out of it are described in classical texts of *Ayurveda*. *Narikela Khanda* is a formulation

which is prepared by using *Narikela* as chief ingredient.<sup>4</sup> *Khanda* is a popular dosage form in *Ayurveda*. *Narikela Khanda granules* is a modified method of *Narikela Khanda*<sup>4</sup> which is prepared in the form of granules by keeping the ingredients same to enhance the stability. *Narikela Khanda* is popularly used to treat *Amlapitta* (hyperacidity).<sup>4</sup> Hence, the study was carried out to study the effect of *Narikela Khanda* and *Narikela Khanda granules* in experimentally induced gastric ulcer in rats and their possible mechanism of action by studying their effects on various mucosal offensive factors like acid-pepsin activity.

### MATERIAL & METHODS

Alcohol (Alembic), Sulphuric acid (CDH), Phenol (CDH), Folin's Reagent (CDH), Sodium Hydroxide (CDH), Topfer's Reagent (CDH) and Phenolphthalein (CDH) were used in the study. All the chemicals or reagents used in the experimental study were procured from standard and reputed firms and are of analytical grade (EXLR) regularly used in the laboratory. All the substances were prepared immediately before use.

### Test drug :

*Narikela Khanda* and *Narikela Khanda granules* were prepared in house laboratory of the department, by following all aseptic measures.

### Animals :

Wister strain albino rats of either sex weighing between 150-250g were selected for the study from the

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TABLE NO. 1 : THE DIFFERENT PARAMETERS STUDIED DURING THE EXPERIMENTS :

	Control (Group A)	N. Khanda (Group B)	N. K. granules (Group C)
Volume (ml/100g)	06.21 ± 00.95	05.21 ± 00.77	03.08 ± 01.12
pH	02.10 ± 00.10	02.20 ± 00.12	02.58 ± 00.08**
Total acidity (mEq/lit)	64.00 ± 12.29	55.00 ± 07.90	31.67 ± 03.07*
Free acidity (mEq/lit)	63.00 ± 10.56	62.50 ± 04.33	38.33 ± 07.37
Peptic activity (µmoles of tyrosine released/ml/min)	09.85 ± 00.86	10.28 ± 01.05	06.96 ± 00.53*
Total protein (mg/dl)	46.00 ± 03.80	41.00 ± 02.10	49.83 ± 03.45
Total carbohydrate (mg/dl)	172.45 ± 36.17	195.36 ± 30.11	170.20 ± 09.36
TC:TP ratio	03.64 ± 00.60	04.09 ± 00.20	03.77 ± 00.23
Ulcer index (mm)	02.40 ± 00.46	01.50 ± 00.41	01.53 ± 00.74

n=6; Data = Mean±SEM; \* p < 0.05; \*\* p < 0.01,

animal house attached to the institute. They were housed at 22±2°C with constant humidity 50-60%, on a 12 hour natural day and night cycles. They were fed with diet Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water was given *ad libitum*. The experiments were carried out in accordance with the directions of the Institutional Animal Ethical Committee (IAEC).

#### Dose selection & schedule :

The dose of the trial drugs were calculated by extrapolating the human dose to animals based on the body surface area ratio by referring to the standard table of Paget & Barnes (1969). The test drugs were dissolved in tap water and administered orally at morning hours.

#### Experimental procedure :

The animals were divided in to three groups of 6 animals each. Out of three groups, group A is taken as normal control and received water, while group B and C groups receiving *Narikela Khanda* and *Narikela Khanda granules* respectively at the dose of 1.1 gm/kg for 7 days. Pylorus ligation was carried out on 18 hours fasted rats on eighth day following the method of Shay *et al.* (1945)<sup>5</sup>. After six hours the animals were sacrificed by over dose of ether. The stomach was cut open along the greater curvature and the gastric juice was collected, centrifuged and subjected to biochemical analysis. Total and free acid was estimated as described by Varley (1962), peptic activity by Debnath P.K. *et al.* (1974)<sup>6</sup>. Total carbohydrate content by Nair (1976). Total protein by Lowery O. H. *et al.* (1951)<sup>7</sup>. TC:TP ratio was calculated as per the method of A.K.Sanyal (1983)<sup>8</sup>. The volume of the supernatant was expressed as ml/100g body weight and pH of gastric juice was also recorded. The stomach was washed with normal saline and the lesions were observed using a magnifying glass and the ulcer index was determined<sup>9</sup>.

#### Statistical analysis :

The results are presented as Mean±SEM. The data generated during the study were subjected to student's 't' test for unpaired data to assess the statistical significance. The values were considered significant at the levels of p<0.05, p<0.01 and p<0.001.

#### RESULTS

*Narikela Khanda* did not showed any significant effect on gastric juice volume, pH of gastric juice, total acidity, free acidity, peptic activity, total protein, total carbohydrate, TC:TP ratio and ulcer index where as *Narikela Khanda granules* significantly increased pH of gastric juice and significantly decreased total acidity and peptic activity (Table -1).

#### DISCUSSION

*Narikela Khanda* is a compound herbal formulation which is indicated in treatment of *Amlapitta*<sup>4</sup>. *Narikela (Cocos nucifera* Linn.) is a major component of this formulation. Formulation factors also play important role in the therapeutic acceptance of a putative drug. An effective drug can be rendered in-effective by faulty formulation. Many factors are required to be taken in to consideration while formulating like patient acceptability, impact of formulation on pharmacokinetics and pharmacodynamics. *Narikela Khanda*, as mentioned above, is indicated in classics for the treatment of *Amlapitta* which can be roughly equated with hyperacidity related disorders. Our team was interested to make it more patient friendly hence it was thought worthwhile to explore the possibility of administering the *Narikela Khanda* in the form of granules. As it is a well known fact that converting the raw drugs in to any formulation influences the efficacy of a test drug. So, the study was undertaken to compare the pharmacological evaluation to ascertain whether administration of *Narikela Khanda* in *granules* form lead to change in pharmacological activity or not.

Gastric ulceration is caused by many factors like stress, alcohol, drugs etc. and being the most prevalent gastrointestinal disorder it is considered as a major therapeutic target<sup>10</sup>. Large numbers of drugs ranging from proton pump inhibitors, H<sub>2</sub> receptor antagonists to cytoprotective agents are being used for treatment of this disease. However a rational therapy for this disease still remains elusive and search for safer potential drug is being attempted world over. The development of gastric ulcers occurs with imbalance of acid-pepsin (offensive) and the breakdown of mucosal barrier (defensive). Local mechanisms implicated in mucosal defense are, mucus like alkaline secretions, rich mucosal blood flow, mucosal hydrophilic activity, rapid epithelial cell renewal, mucosal sulphahydryls and increased resistance of gland cells in deep mucosa to acid and peptic activity. Calcium ions are involved in the regulation of acid secretion in the stomach<sup>11</sup>.

Stomach plays an important role in the digestion of the ingested food besides functioning as a temporary food store. The digestive process is initiated by acidification of ingested food, which would provide optimal condition for activation of enzyme pepsin. Gastric secretion occurs in stages involving cephalic, gastric and intestinal phases which are influenced by many complex neuronal and hormonal interactions. Gastric juice is a multi component secretion containing different constituents contributed by different cells of glandular mucosa. The parietal (oxyntic) cells secrete HCl. The chief cells secrete pepsinogen, which is converted to pepsin, which can produce powerful proteolytic effect on gastro-duodenal mucosa. Normally the stomach mucosa is protected from the auto-digestive effect of acid pepsin by a very efficient mucosal protection mechanism. It includes secretion of mucus, bicarbonate and formation of protective barrier. Gastric ulceration occurs wherever there is imbalance between the aggressive acid-pepsin and defensive mucus secretion<sup>12</sup>.

Several factors modulate both acid secretion and mucus barrier formation. Histamine plays important role (through H<sub>2</sub>-receptor) in acid secretion. Parietal cells contain receptors for both muscarinic cholinergic agonists and gastrin. Stimulation of H<sub>2</sub>-receptor activates adenylyl cyclase, which promotes formation of cyclic AMP and in addition Ca<sup>++</sup> entry into the cells is also enhanced. These are two effects which cause activation of H<sup>+</sup>K<sup>+</sup> ATPase enzyme which catalyses the exchange of intracellular H<sup>+</sup> ions for extracellular K<sup>+</sup> with concomitant increase in the permeability of apical membrane to K<sup>+</sup> and Cl<sup>-</sup><sup>13</sup>.

Combination of histamine and acetylcholine with their respective receptors on parietal cells causes

increased activity in it. The fundic mucosa contains cells that are capable of releasing somatostatin and serotonin also. Somatostatin acts as a potent inhibitor of gastric secretion. Cholecystokinin (CCK) secretion of which is enhanced by the presence of fat in the duodenum and secretin whose secretion is enhanced by presence of acid in duodenum acts as gastric secretion inhibitors as well as inhibitors of gastric motility<sup>13</sup>.

Mucus produced by the gastric glands form a thin protective layer over the entire mucosal surface of the stomach virtually preventing the contact of acid pepsin with the mucosal layer. Bicarbonate secreted by the surface epithelial cells, which remains beneath and within the mucosal layer, further contributes to antacid property of the mucosal barrier. Increased blood flow to the gastric mucosa enhances the removal of acid from the mucosa. Faster regeneration and removal of mucosal cell also helps in the proper maintenance of protective mucosal barrier<sup>14</sup>. From these resume it becomes clear that any factor, which promotes aggressive factors and weakens defense mechanism leads to ulcer formation.

In pylorus ligation model, it has been postulated that the digestive effect of accumulated gastric juice and interference with gastric blood circulation are responsible for the induction of gastric ulcer (Brodie, 1966; Patel *et al.*, 2000). Enhanced secretion of acid-pepsin leading to auto digestion of gastric mucosa and break down of mucosal barrier is also responsible for gastric ulcers (Goel *et al.*, 1991). Stimulation of pepsin secretion, with or without secretion of acid, is the major factor in the development of gastric ulcers (Gaw *et al.*, 1995; Anup *et al.*, 2003).

**Volume of gastric juice :** Volume of secretion of gastric juice is an important factor in the formation of ulcer due to the exposure of the unprotected lumen of the stomach to the accumulating acid. If a formulation is to be effective against the gastric ulcer it should have one or more than one of the following attribute: it should have acidity decreasing effect. Acidity can be decreased by either anti-secretory effect that is to reduce the gastric juice secretion or the drug should neutralize the gastric acidity<sup>15</sup>. The results obtained show that the test formulations did not modify gastric secretion to significant extent. In fact, in Group B around 17% decrease was observed which is statistically non significant. In Group C, 50% decrease was observed in volume of gastric juice which is statistically non-significant. This may be due to enhancing the function of oxyntic cell and inhibiting the function of chief cells and mucus secreting cells. This clearly shows presence of

some anti-secretory effect in the drug. *Narikela Khanda granules* were found to be more effective in this regard in comparison to *Narikela Khanda*.

*pH of gastric juice* : *Narikela Khanda* and *Narikela Khanda granules* reduced the pH of Gastric juice secretion in pyloric ligated rats. The observed increase of gastric juice pH in the *Narikela Khanda granules* treated group is statistically significant, which is dependent upon gastric acidity. This shows that *Narikela Khanda granules* can neutralize gastric acidity to significant manner or also may have its inhibitory effect on acid secretion.

*Free acidity of gastric juice* : Normally the free acidity of the resting contents lies between 1.5 to 2.0 mEq of HCl. After the food is taken the acidity is reduced by dilution. The free acidity then steadily rises and becomes maximum 40-50mEq of HCl in the second hour. Then it gradually declines. In gastric ulcer the value increases up to 3 times. Both the formulations failed to produce any significant effect on this parameter.

*Total acidity of gastric juice* : Total acidity is the sum total of free HCl, organic acids, combined acid and acid salts. *Narikela Khanda granules* produced significant reduction in total acidity.

*Peptic activity of gastric juice* : Modulation of the peptic activity is the next mechanism to be considered. Data pertaining to peptic activity reveals that *Narikela Khanda* treated group showed marginal and statistically non-significant increase in peptic activity in comparison to control. In *Narikela Khanda granules* statistically significant decrease in peptic activity was observed in comparison to pyloric ligated rats. This clearly rules out anti-peptic activity of *Narikela Khanda* as the mechanism of anti-ulcer effect. The reason for elevation of peptic activity may be increased release of the precursor molecular from the peptic cells in the gastric glands. It has been also observed [Chaudhary, S.K.-1997] that calcium given orally can cause gastric stimulation. The observed increase may be due to the calcium content in the formulation. From the data generated during the study it can be inferred that *Narikela Khanda* has no inhibitory effect on pro-ulcer aggressive factor. This implies that the observed anti-ulcer effect in *Narikela Khanda* may be due to enhance defense mechanism. In *Narikela Khanda granules*, statistically significant decrease in peptic activity was seen. This anti-peptic activity of *Narikela Khanda granules* can be the reason for anti-ulcer effect.

*Total protein of gastric juice* : As enumerated above increased secretion of mucus, and polymerization of the mucopolysaccharides, increased blood supply, increased regeneration of gastric epithelial cells, increased bicarbonate secretion, increased prostaglandin and somatostatin are among the important defense mechanism, which provide cytoprotection to gastric mucosa against the effect of aggressive factors. In ulcers there is leakage of plasma protein in to gastric juice. This reflects the increased protein concentration in the gastric juice<sup>16</sup>. Present study shows both the formulations did not-produced any significant effect on total protein content of gastric juice.

*Total carbohydrate of gastric juice* : Important factor in ulcer is the deficiency of mucosa to resist the aggressive acid-pepsin activity. The status of mucosal barrier can be studied by determining the concentration or output of carbohydrate. Carbohydrate content of the gastric juice and increased carbohydrate is considered as the index of the extent of mucus content of the gastric juice<sup>17</sup>. As could be observed from the data it is revealed that test formulation *Narikela Khanda* produced moderate but statistically non-significant increase in total carbohydrate content, whereas *Narikela Khanda granules* showed no difference in total carbohydrate content. *Narikela Khanda* enhanced carbohydrate content of the gastric juice which indicates that the anti-ulcer activity of the formulation may also be due to increased mucus secretion. There may be also a possibility that the anti-ulcer activity of the *Narikela Khanda* may be due to increased blood supply to the gastric mucosa, enhancement of gastric epithelial cells turn over or increased secretion of bicarbonate.

*TC : TP ratio of gastric juice* : This factor is related to enhancement of defense mechanism against acid-peptic activity. Normally total carbohydrate (TC) content of the gastric juice is considered to be an index of mucosal secretion. Hence TC, total protein (TP) content of the gastric juice is estimated to calculate TC: TP ratio. This ratio increases with decrease in the secretion.<sup>15</sup> Both the formulations did not affected this factor to significant extent.

## CONCLUSION

From the present experimental study it can be concluded that the test formulations in the mentioned dose possess anti-ulcer activity against pyloric ligation induced gastric ulcers which are mainly dependent on acid-pepsin activity. The data generated during this study clearly indicates that administration of *Narikela Khanda*

*granules* for the treatment of gastric ulcer condition of different aetiology would be useful and this formulation retains the efficacy of the classical *Narikela Khanda*.

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## हिन्दी सारांश

### नारीकेल खण्ड एवं नारीकेल खण्ड ग्रेन्युल्स का एन्टी अल्सर क्रियात्मक अध्ययन

प्रमोद सी. बारगी, अशोक बी. के., बी.जे. पाटगिरि, पी. के. प्रजापति एवं बी. रविशंकर

आज के आधुनिक काल में अम्लपित्त एवं अल्सर जैसे उदर रोग एक प्रायः चिकित्सकीय विषयवस्तु हैं। ये बहु क्रियात्मक निदानों से युक्त एक प्रमुख स्वास्थ्य समस्या है। आजकल एक ऐसी क्रियात्मक काष्ठौषधि कल्पना की जरूरत है जो अम्लपित्त का निवारण कर सके। प्रस्तुत तुलनात्मक प्रायोगिक अध्ययन, एल्बिनो जातीय मूषको में पैलोरिक लाइजेशन द्वारा गेस्ट्रिक अल्सर उत्पन्न कर नारीकेल खण्ड एवं नारीकेल खण्ड ग्रेन्युल्स के काष्ठौषधिय संयुक्त कल्पना की अल्सर प्रतिरोधात्मक क्रियाशीलता देखने के लिये किया गया। दोनों औषधियाँ का तुलनात्मक प्रभाव गेस्ट्रिक रस का वोल्युम, पि. येच, टोटल एवं फ्री एसिड, पेप्टिक क्रिया, संपूर्ण कार्बोहाइड्रेट एवं संपूर्ण प्रोटीन जैसे प्रक्रियाओं पर किया है। दोनों औषधियाँ एन्टी अल्सर कार्य में प्रभावी हैं परन्तु अपेक्षाकृत नारीकेल खण्ड ग्रेन्युल्स का एल्बिनो जातीय मूषको में अच्छा प्रभाव मिला है।

