

In Vitro Anti-Ulcer Activity Of Vempam Poo (Flower Of Azadirachta Indica) Ooral Kudineer – Siddha Herbal Drug

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ABSTRACT

Introduction and Aim: Peptic ulcer disease (PUD) is prevalent among population. It is a global problem with a lifetime risk of development ranging from 5% to 10%. In present day, global population evolution towards herbal remedies for complete healing of disease. Many herbs have better remedies for the management of peptic ulcer. Siddha system is one of the traditional medicine in India, which is helpful in treating the disease by using herbs, metals, minerals and animal products. Among them, flower of *Azadirachta indica* (*Vempam poo*) *ooral kudineer* is indicated for ulcer (*Gunmam*) in Siddha literature. The aim of the study is to evaluate the Anti ulcer activity of *Vempam poo ooral kudineer* (VOK).

Material and Methods: Anti-ulcer activity was assessed using *in vitro* method of Acid neutralizing capacity and H^+/K^+ - ATPase inhibition activity.

Results: The result revealed that the extract of VOK at concentration 100mg, 500mg, 1000mg showed a significant reduction in acid neutralizing capacity (ANC), i.e., 124, 12.3, 10.75 respectively, as compared to standard $Al(OH)_3 + Mg(OH)_2$ (500 mg) which is 15. VOK significantly inhibits the enzyme H^+/K^+ - ATPase, which is responsible for the secretion of acid and effect was comparable to omeprazole.

Conclusion: The study concluded that the *Vempam poo ooral kudineer* (VOK) has anti ulcer activity by acid-neutralizing capacity (ANC) and inactivation of H^+/K^+ ATPase.

Keywords: Siddha medicine, *Ooral kudineer*, *Azadirachta indica*, Anti-ulcer, *Vempam poo* (Neem flower)

INTRODUCTION

In this expeditious world, Peptic Ulcer is one of the commonest problems seen among the world population. Peptic Ulcer Disease (PUD) is a global problem with a lifetime risk of development ranging from 5% to 10% (1, 2). Peptic ulcers are acid-induced lesions found in the stomach and duodenum characterized by denuded mucosa with the defect extending into the submucosa or muscularis propria (3). In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain, and cramps along with vomiting blood (4). The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) (5). Peptic ulcer disease (PUD) has various causes includes *Helicobacter pylori*-associated PUD and NSAID-associated PUD account for the majority of the disease etiology (6). Eating spicy food substances increase the symptoms of ulcers; the other symptoms include nausea, vomiting, and weight loss. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur even in the complete absence of acid (7). The prevalence of Peptic ulcers is increasing among the population due to the unhealthy food habits of the people (8). Recently, considerable attention has been paid to utilize plant based products for the prevention and cure of various diseases. It is documented by WHO that 80% of the world's population has faith in traditional medicine, particularly plant drugs for their primary healthcare (9).

In Siddha literature, Sage Yugi the founding Father of Siddha pathology, has classified the conditions of acute and chronic ulcer into eight broad types under the title '*Gunmam*' which means the patient doubles up in abdominal pain and depressed. It is said to be a result of disequilibrium of three humors viz. *Vatham* (Gas energy), *Pitham* (Heat energy) and *Kabam* (Water energy) due to dietary reasons (10). Herbs play a major role and first line of medicine in the Siddha treatment protocol. Many herbs were used as remedies for anti-ulcer. *Azadirachta indica* (*Neem*) is a commonest plant present all over the Tamil Nadu. It belongs to *meliaceae* family and has several medicinal values. The leaves, flowers, seeds, roots and bark of the plant possess different phytoconstituents. The bitter principles of *Azadirachta indica* are also known to increase the flow of saliva and gastric juice as a result of which the plant is used as stomachics (11). *Azadirachta indica* lowered blood glucose level and attenuated gastric ulcerogenesis (12). In Siddha, the flower of *Azadirachta indica* (*vempam poo*) *oral kudineer* is indicated for ulcer (*Gunmam*).

Kudineer (Decoction) also named as *Kiyazham*, *Kashayam*, *Unneer*, and *Marundhu neer* (13). The shelf life of this form of medicine is one *saamam* (three hours) and hence it should be consumed within the specified period from the time of preparation (14). There are two types of *Kudineer* viz. *Ooral kudineer* (prepared after soaking the contents for 2-12 hours or overnight) and *Kodhi Kudineer* (prepared by boiling). The *kudineer* is used in external wash, gargling, drinking, wound cleaning, and purgation. Decoctions can be used both internally and externally according to the disease condition. As a whole, decoctions are waterbased extracts of either herbal, herbo-mineral or animal-based ingredients which are easily absorbed into the body and enter into the blood stream rapidly for better efficacy (15).

Among them, *oral kudineer* is one of the internal medicine, defined as dilute liquid extract obtained from a drug or drugs by soaking it in water (cold or hot) (16). The objective of this present study was undertaken to evaluate the Anti ulcer activity of *Vempam poo oral kudineer* (VOK) by *invitro* method.

MATERIAL AND METHODS

Sample collection

The Flower of *Azadirachta indica* (*vempam poo*) used in this study was collected during the month of April/May (2023) month from campus of National Institute of Siddha, Chennai. Tamilnadu, India and authenticated by Botanist, National Institute for Siddha, Chennai (NISMB6332023). The collected flowers were dried and stored in air tight container.

Preparation of Vempam poo Ooral Kudineer (VOK)

4.2 g (1 *varagan*) of Flower of *Azadirachta indica* (*Vempam poo*) was taken and soaked in 90 ml (3 ounce) of hot water for 2 hours. Then it was filtered and the extract was used for analysis (17).

In-vitro evaluation of Antiulcer Activity

Acid Neutralizing Capacity

The acid-neutralizing capacity of aqueous extract of VOK was evaluated at 100mg, 500mg, and 1000mg doses. The combination of aluminum hydroxide and magnesium hydroxide (500mg) was taken as standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1.0 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed.

With 0.5N Sodium hydroxide, the excess HCl was immediately titrated until the pink colour is attained (18).

The moles of acid neutralized is calculated by, Moles of acid neutralized = (Vol. of HCl × Normality of HCl) - (Vol. Of NaOH × Normality of NaOH)

Acid neutralizing capacity (ANC) per gram of antacid = moles of acid neutralized divided by Grams of Antacid/Extract.

H⁺/K⁺ - ATPase Inhibition Activity

Preparation of H⁺/K⁺ - ATPase Enzyme

To prepare H⁺/K⁺ - ATPase enzyme sample, the fresh goat stomach was purchased from the local slaughter house, the gastric mucosa of the fundus was cut-off and opened, the inner layer of the stomach was scrapped out for the parietal cell. The parietal cell obtained from the stomach was homogenized in 16mM Tris buffer with pH of 7.4, which has 10% Triton X-100 and centrifuged at 6000 rpm for 10mins. After centrifuged the supernatant solution was used for the H⁺/K⁺ - ATPase inhibition.

Assessment of H⁺/K⁺ ATPase inhibition

The reaction mixture containing 0.1 ml of enzyme extract (300 µg) and extract VOK at different concentrations (20µg, 40µg, 60µg, 80µg, 100µg) was pre-incubated for 60 min at 37°C. The reaction was initiated by adding substrate 2 mM ATP (200µL), in addition to this 2mM MgCl₂ (200µL) and 10mM KCl (200µL) was added. After 30 min of incubation at 37 °C the reaction was stopped by 4.5% ammonium molybdate, and 60% perchloric acid was added and centrifuged at 2000rpm for 10 min, and in spectrophotometrically inorganic phosphate was released and measured at 660nm by following the FiskeSubbarow method. Briefly, at 10 min at room temperature, 1ml of supernatant 4ml of Millipore water, 1ml of 2.5% of ammonium molybdate, 0.4ml of

ANSA was added. At 660nm inorganic phosphate, absorbance has been measured at various doses of the extract; the enzyme activity has been calculated as micromoles of Pi released per hour (19). Results were compared with the known anti-ulcer PPA inhibitor Omeprazole.

% enzyme inhibition has calculated using the formula:

Percentage of inhibition = $[\text{Activity (control)} - \text{Activity (test)}] / \text{Activity (control)} \times 100$

RESULTS

Acid Neutralizing Capacity

The neutralizing effect of VOK was studied for concentration (100mg,500mg,1000mg) and standard Aluminium Hydroxide + Magnesium Hydroxide $[\text{Al}(\text{OH})_3 + \text{Mg}(\text{OH})_2]$ (500mg). The results obtained envisage that the extract VOK at concentration 100mg, 500mg, 1000mg showed a significant reduction in acid neutralizing capacity (ANC), i.e., 124, 12.3, 10.75 respectively, as compared to standard $\text{Al}(\text{OH})_3 + \text{Mg}(\text{OH})_2$ (500 mg) which is 15. The extract of VOK at a concentration of 500 mg has been found to neutralize acid significantly as compared to standard. The results have been shown in table 1 and figure 1

Table 1: Effect of Aqueous Extract of VOK of on Acid Neutralizing Capacity

S. No	Concentration (mg)	Volume of NaOH consumed (ml)	mEq of Acid Consumed	ANC per gram of Antacid
1.	100mg VOK	35.2	12.4	124
2.	500 mg VOK	47.7	23.85	12.3
3.	1000 mg VOK	38.5	19.25	10.75
4.	500 mg $[\text{Al}(\text{OH})_3 + \text{Mg}(\text{OH})_2]$	45	7.5	15

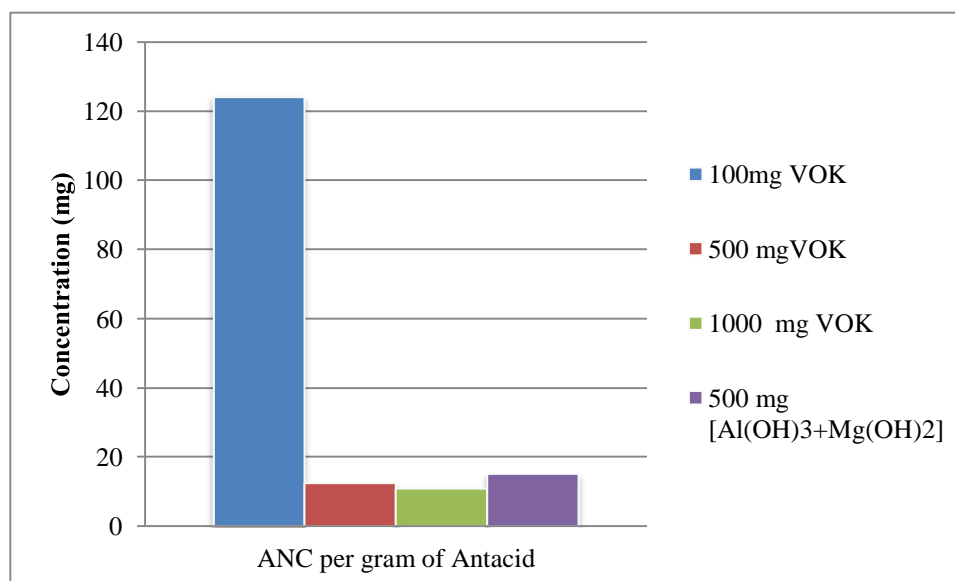


Fig. 1: Effect of Vempam Poo Oral Kudineer on Acid Neutralizing Capacity

H⁺/K⁺ - ATPase Inhibition Activity

The H⁺/K⁺ - ATPase inhibition activity of Vempam poo oral kudineer (VOK) extract at a various concentration (100µg, 200µg, 300µg, 400µg, and 500µg) has been compared with Omeprazole as same concentration (100µg, 200µg, 300µg, 400µg, and 500µg) considered as standard. The extract significantly showed activity in a dose dependent manner. Maximum percentage inhibition of has been observed for extract VOK 57.14% at a concentration of 500µg is and standard Omeprazole showed 71.43 %. The results have been tabulated in Table 2 and figure 2.

Table 2: Effect of Vempam Poo Oral Kudineer on H⁺/K⁺ - ATPase Inhibition Activity

S.NO	Concentration ((µg)	Standard Omeprazole	Sample VOK
1	Control	0.00	0.00
2	100	25.00	14.29
3	200	35.71	25.00
4	300	42.86	32.14
5	400	57.14	42.86

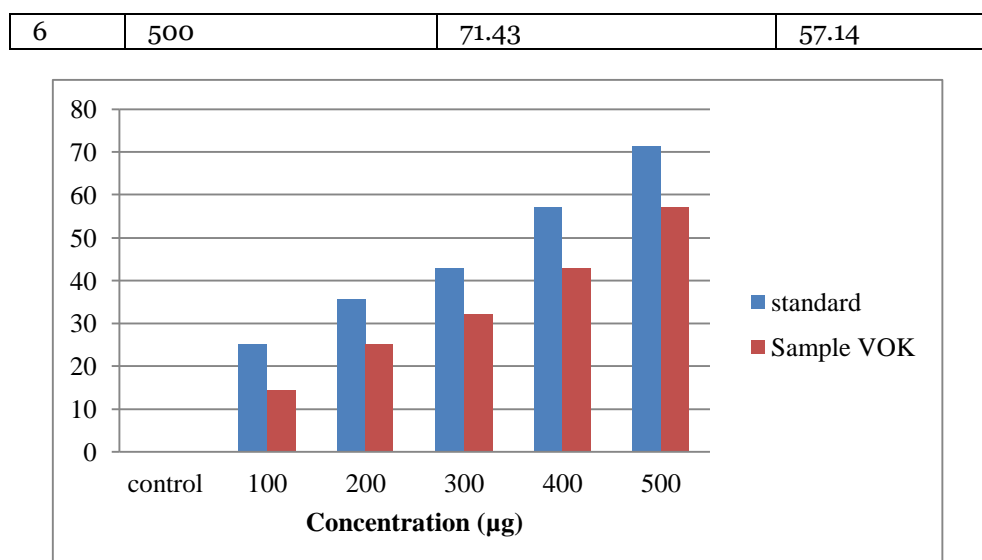


Fig. 2: Effect of Aqueous Extract of VOK of on H⁺/K⁺ - ATPase Inhibition Activity

DISCUSSION

Azadirachta indica (Neem) is one of the most common medicinal plants that grow all over India. Neem has a role in the treatment of disorders like microbial infections, skin diseases, dental disorders, malaria, syphilis, leprosy and has antiseptic property (20-22). Anti-inflammatory, immunostimulant and antiulcerogenic activity (23-25). Flavanoids in *A. indica*, like rutin and quercetin have been reported to possess antiulcer and anti-inflammatory activities (26).

Peptic ulcers results due to imbalance between offensive (acid, pepsin, and *H. pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) (10). Excessive secretion of gastric acid or stomach acid (i.e., HCl), inflames the stomach lining and produces ulceration (27). Antacids act by neutralizing gastric acid and thereby reduce the gastric pH. The regain balance is maintained for the use of therapeutic agents differently for the use of gastric acid secretion inhibition or by increasing the mucosal production to boost the mucosal defense mechanism by stabilizing the surface epithelial cells or inhibition of prostaglandin synthesis (28).

In Siddha system of medicine treating and managing acid peptic disease is done in natural ways i.e., use of natural drugs, diet and lifestyle changes. The concept of Siddha medicine "Food is medicine, Medicine is food" explains the same (29). *Azadirachta indica* flower (*Vempam poo*) is bitter in taste, stimulant, stomachic action and indicated for vomiting, *vatha* diseases, worm infestation, loss of taste and for dryness of mouth.

Previous studies on shade dried flower of *A. indica* showed the presence of higher amount of protein, fibre and volatile oil as sesquiterpenes, caryophyllene, and caryophyllene oxide which was effective against inflammatory diseases (30). Neem bark extract blocks acid secretion by inhibiting the activity of the H⁺/K⁺-ATPase as does by omeprazole. It has potential for therapeutic application as a preventive measure for hyperacidity in any form of drug therapy. Since H⁺/K⁺-ATPase acts as a terminal proton pump for controlling acid secretion (31), leaf extract appears to block acid secretion at least through the inhibition of H⁺/K⁺-ATPase activity of the parietal cell. A number of compounds have already been isolated from Neem leaf (32). Nimbidine, an antiulcer terpenoid isolated from Neem seed kernel seems to be less active than the crude leaf extract as the former shows antiulcer Mechanism of antiulcer effect of Neem leaf extract 173 activities above 20 mg/kg dose (33). In folk medicine, flower of *Azadirachta indica* decoction is used as an herbal tea for improper digestion.

In vitro method of the acid-neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize, and it has been measured by a process known as back titration. In ANC, the extract of VOK at 500mg concentration showed a significant reduction in ANC of 12.3. H⁺/K⁺ ATPase is a key enzyme in inducing acidity; in this study, the ability of VOK to inhibit H⁺/K⁺ ATPase in vitro isolated from goat stomach was studied. Enzyme H⁺/K⁺ ATPase is an important enzyme system located on apical secretory membrane of parietal cell (34). In this study, dose-dependent inhibition of enzyme by omeprazole and extract VOK was observed, suggesting that the *Vempam poo ooral kudineer* (VOK) was significantly able to inhibit enzyme H⁺/K⁺ ATPase, responsible for the secretion of acid and effect was comparable to omeprazole.

CONCLUSION

From this study, It is concluded that the *Vempam poo ooral kudineer* (VOK) has a significant acid-neutralizing capacity (ANC) and inactivation of H⁺/K⁺ ATPase is the major gastroprotective mechanisms of action, which indicates its protective role against inhibiting gastric proton pump. Hence *Vempam poo ooral kudineer* has

anti ulcer activity. This is a preliminary *invitro* study and further *invivo* studies have to be conducted to ensure the efficacy in animals and humans. This drug is easy to prepare and easily over table.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest in this study.

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