NEEM AND RANITIDINE IN COMBINATION PRODUCE SYNERGISTIC AND GASTROPROTECTIVE EFFECTS IN EXPERIMENTALLY INDUCED GASTRIC ULCERS IN MICE

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ABSTRACT

Peptic ulcer is one of the common diseases affecting the mankind. Many studies reveal co-occurrence of both peptic ulcer and hypertension in humans. So, it might be beneficial to choose an optimal drug that will target both these conditions. Azadirachta indica has many chemical constituents that provides protection against a number of disease models. However its effect on gastric ulcers has not been studied. The present investigation was undertaken, to study the gastro-protective and synergistic potential of Neem in combination with ranitidine in experimentally induced gastric ulcers in mice. Neem (20, 40, 80 and 160 mg/kg) was tested for gastric secretion and antiulcer activity in different groups of Mice. Gastric secretion and acidity studies were performed in pylorus ligated mice. Treatment with Neem, resulted in a significant decrease in the amount of gastric secretion, and total acidity and significantly ($P<0.01$), reduced the gastric lesions. It is evident that ranitidine at a dose of 15 mg/kg does not possess significant ulcer protective property. The same result were observed with Neem at doses of 20 mg/kg and 40 mg/kg. However, when two drugs in the aforementioned doses are combined, significant results are obtained. Thus, it may be concluded that ranitidine and NLE in combination produce synergistic effects. The histological changes were also significantly ($P<0.01$) inhibited by Neem. Neem showed significant antiulcer and gastroprotective activity against experimentally induced gastric ulcers. The gastroprotective effects of Neem may be due to its anti-secretory, antioxidant and anti-inflammatory action.

Keywords: Aspirin, Gastric secretion, Gastroprotective, Ranitidine.

I. INTRODUCTION

Peptic ulcer disease encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. The pathophysiology of ulcer involves an imbalance between offensive (acid, pepsin, *H. pylori* and non-steroidal anti-inflammatory agents) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Integrity of gastro-duodenal mucosa is maintained through a homeostatic balance between these

aggressive and defensive factors. Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second re-enforcing gastric mucosal protection. Aspirin is a potent non-steroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin is a major problem.

Peptic ulcer is one of the commonest diseases affecting the mankind. They are so common in industrialized nations. Peptic ulcer disease is the most prevalent gastrointestinal disorder [1]. It is to be characterized as deep lesions that penetrate through the entire thickness of the gastrointestinal tract (g.i.t) including mucosa and muscularis mucosa that develop due to exposure high gastric juice secretions to stomach. The most prominent cause of peptic ulcer is infection with the bacterium called Helicobacter pylori(H. pylori) and the use of drugs like Non-steroidal Anti-Inflammatory Drugs (NSAIDs) (aspirin and ibuprofen) [2]. It is accepted that ulcer occur due to imbalance between offensive acid-pepsin secretion and defensive factors which include mucin-bicarbonate secretion, life span of cells, cell proliferation, mucosal blood flow, mucosal glycoproteins and sulfhydryl compounds. Various factors that play a pivotal role in the pathogenesis of ulcerations like sedentary life style, alcohol intake, spicy food, NSAID and various bacterial infections like *H. pylori* [3]. When patients taking NSAIDs were excluded [3]. Aggressive acid secretion has been reported to play a progressive role in gastric ulceration [4]. Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E2 (PGE2) content[5], and pro-inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)-[6,7] play important roles in the genesis of gastric mucosal damage, and its subsequent development[8,9]. It has been reported that increases in NO synthase (NOS) activity is involved in the gastrointestinal mucosal defense and also in the pathogenesis of mucosal damage [10, 11]. Integrity of gastro duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors .Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second re-enforcing gastric mucosal protection [12, 13]. Currently available antiulcer medications provide only pain relief and healing of ulcers but no drug prevents ulcer recurrence. So the search for newer medications which will effectively reduce complications and prevent relapse is greatly needed.

Azadirachta indica commonly known as Neem, is native of India and naturalized in most of tropical and subtropical countries are of great medicinal value and distributed widespread in the world. Medicinal plants have been reported to have antioxidant activity [14]. Different parts of the plant have been reported to possess medicinal properties like hypoglycemic, antiseptic, wound healing of skin diseases and antiulcer activities. Pillai [15] reported the ulcer protective effects of nimbidin, the active principle obtained from Neem seed oil and the bark of Neem tree, in these histamine-induced lesions in guinea pigs**.** Garg [16] again demonstrated the antiulcer activity of Neem leaves in stress induced and in ethanol induced gastric ulcer in Albino mice. An aqueous extract of Neem bark has been shown from our laboratory to possess highly potent antacid secretory and antiulcer activity and the bioactive compound has been attributed to a glycoside [17].

II. MATERIALS AND METHODS

The Chemicals used are as follows:

2.1) Chemicals

2.1.1) Cerboxymethyl cellulose

2.2) Drug

2.2.1) Neem leaf extract

2.2.2) Ranitidine

2.2.3) Aspirin

2.2.4) Anaesthetic ether

2.3 Experimental Laboratory animals: Swiss Albino mice Swiss Albino mice, weighing around 30-35g of approx. 8 weeks old, were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Reg. No. 1129/bc/07/CPCSEA). The research work was approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. IAEC/2013/1E (13/08/2013). Food and water to mice were provided ad libitum (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups. The mice were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22± 2^0 C with 12 h light/dark cycle. Swiss albino mice was selected as the experimental animals, because of:

a) Their physiological activity is almost similar to that of man (as 90% of their genes are similar to humans).

b) Rapid rate of inbreeding.

c) Small size.

d) Early puberty (sexual maturity).

e) Short gestation period.

III. METHODS

This study will mainly focus upon Gastroprotective effects of NLE (Neem leaf extract) in aspirin induced ulcers and pyloric-ligated ulcers in albino mice in order to throw further light on synergistic effect of Neem and ranitidine in experimentally induced gastric ulcers in mice.

Work plan:

The study will be carried out in following parts:-

2.4.1) Preparation of NLE (Neem leaf extract).

a) Induction of ulcer by aspirin

b) Calculation of ulcer index. (Ulcer index=10/X. where X = total mucosal area/total ulcerated area.)

2.4.2) Effect of NLE on ulcer -

a) The ulcer healing properties of Neem leaf extract will be observed by ulcer index method. [Ulcer index=10/X (where X= total mucosal area/total ulcerated area]

b) The comparative assessment of Neem leaf extract with the known H2 blocker-ranitidine by ulcer index

method. [Ulcer index=10/X (where X = total mucosal area/total ulcerated area.]

IV. RESULTS

- The study was concerned with observing the synergistic and Gastroprotective effects of NLE+ ranitidine on volume of gastric secretion in pyloric ligated mice. Ranitidine served as the standard drug.

Table-1

Effect of combination of ranitidine and NLE on volume (in ml) of gastric secretion in pyloric-ligated mice

	Control	Ranitidine (15mg/kg)	Ranitidine(15mg/kg)+ NLE(20mg/kg)	Ranitidine(15mg/kg)+ NLE(40mg/kg)
Mean	0.666	0.5	0.35	0.225
SE	0.08819	0.03651	0.07188	0.04787

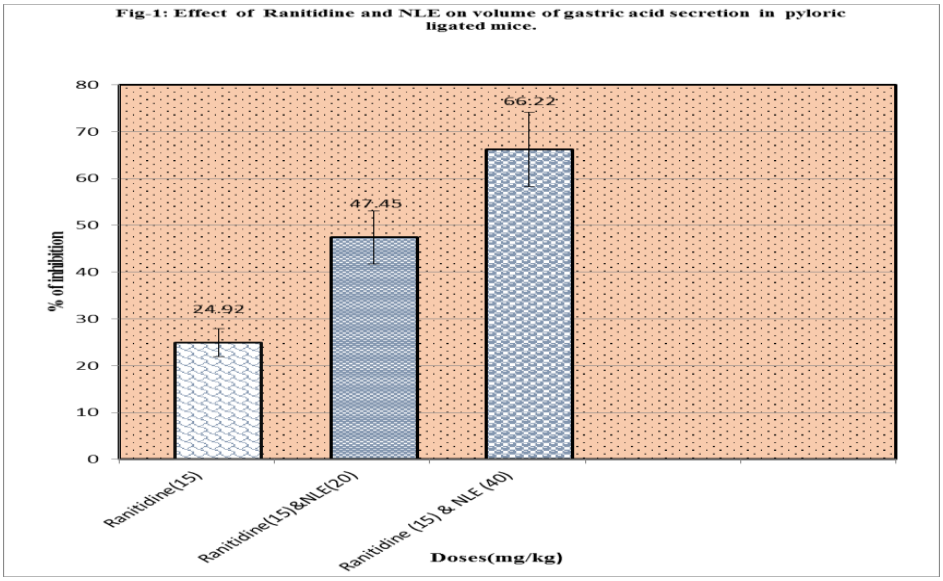
Table-2

Analysis of variance (ANOVA)

Source of Variation	Sum of Square	df	Mean square	F
Between	0.6553	3	0.2184	8.789**
Error	0.4971	20	2.4854E-02	
Total	1.152	23		

df= Degree of freedom

Referring to the table of F, for p =0.01 against 3 df between mean square and 20 df for within mean square, we find a value of 4.94. Since the value 8.789 for F obtained in the present experiment is greater than the recorded value 4.94. Hence the decrease in volume of acid is significant.



- This part of study was concerned with observing the synergistic and Gastroprotective effects of NLE+ ranitidine on ulcer index in pyloric ligated mice. Ranitidine served as the standard drug.

Table-3

Effect of combination of ranitidine and NLE on ulcer index in pyloric ligated mice

	Control	Ranitidine (15mg/kg)	Ranitidine (15mg/kg)+NLE (20mg/kg)	Ranitidine (15mg/kg)+NLE (40mg/kg)
Mean	0.325	0.2783	0.11	0.015
SE	0.03243	0.01276	0.02257	0.00957

Table-4

Analysis of variance (ANOVA)

Source of Variation	Sum of Square	df	Mean square	F
Between	0.3754	3	0.1251	45.95**
Error	5.4467E-02	20	2.7233E-03	
Total	0.4298	23		

df= Degree of freedom

Referring to the table of F, for p =0.01 against 3 df between mean square and 20 df for within mean square, we find a value of 4.94. Since the value 45.95 for F obtained in the present experiment is far greater than the recorded value 4.94. Hence the decrease in ulcer index is significant.

- This part of study was concerned with observing the synergistic and Gastroprotective effects of NLE+ ranitidine on number of ulcers/mice in pyloric ligated mice. Ranitidine served as the standard drug.

Table-5

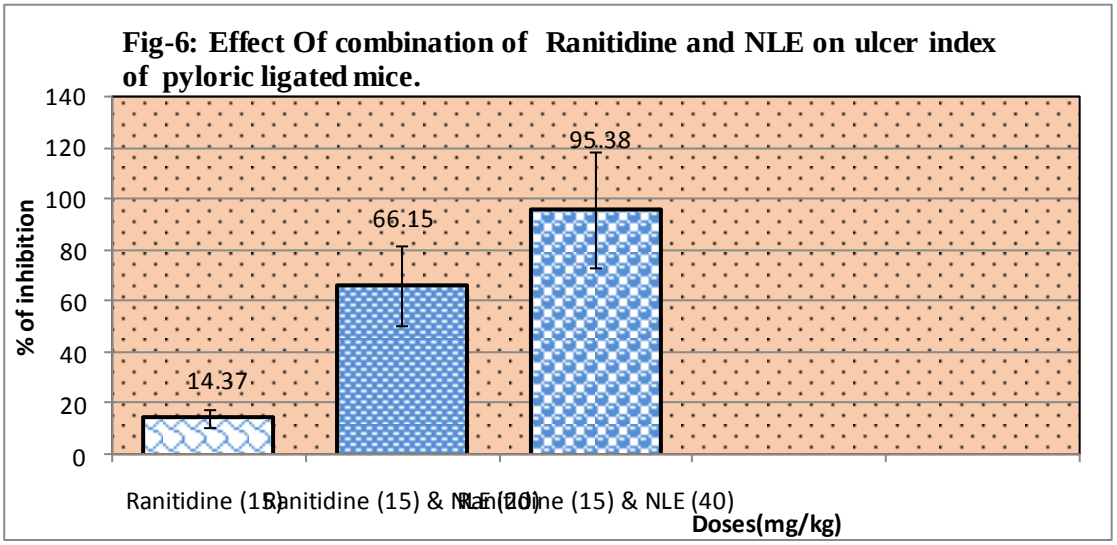
Effect of combination of ranitidine and NLE on number of ulcers/mice in pyloric ligated mice

	Control	Ranitidine (15mg/kg)	Ranitidine(15mg/kg)+ NLE(20mg/kg)	Ranitidine(15mg/kg)+ NLE(40mg/kg)
Mean	6.33	4.5	3.166	2.83
SE	0.71492	0.56273	0.30732	0.30732

Table-6

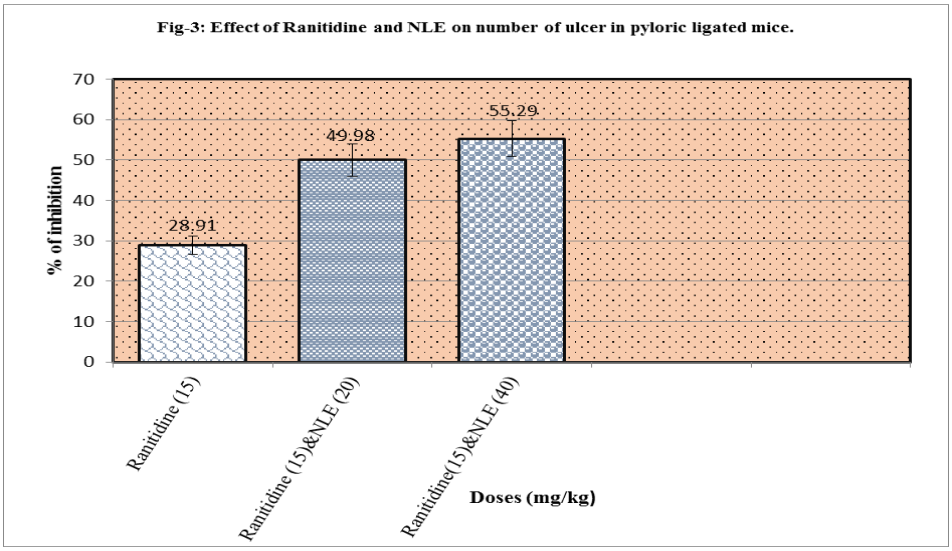
Analysis of variance (ANOVA)

Source of Variation	Sum of square	df	Mean square	F
Between	45.67	3	15.15	9.936**
Error	30.50	20	1.525	
Total	75.96	23		



df= Degree of freedom

Referring to the table of F, for p =0.01 against 4 df between mean square and 25 df for within mean square, we find a value of 4.94. Since the value 9.936 for F obtained in the present experiment is far greater than the recorded value 4.94. Hence the decrease in number of ulcers is significant.



The effect of NLE was compared with that of the H2 blocker ranitidine taken as the standard drug. The total volume of gastric content in the control group was in the range of .2-.5 ml with a mean volume of .333 ml. Pillai *et al*. (1978) have reported control volume of 6.019 ml in their study. It is evident from the foregoing discussion that NLE does not possess significant ulcer protective action below the dose of 80 mg/kg of body weight. It is also observed that the ranitidine in a dose of 15 mg/kg did not exhibit any significant role in reducing gastric secretion and in ulcer healing properties. In an extension of this work, ranitidine (15 mg/kg) with NLE in low doses were combined to observe the occurrence of any additive or synergistic effects. The results of the combined study were compared with that of ranitidine (15 mg/kg). The effects were studied in pyloric ligated mice. Ranitidine in a dose of 15 mg/kg did not produce significant reduction of the volume gastric secretion (Table-1, Fig.-1). As it was observed, low doses of NLE also lacked significant anti-secretory effect. However, when 15 mg/kg of ranitidine was administered in combination with graded doses of NLE(20 and 40 mg/kg), a progressive decline in the volume of gastric secretion was observed. The mean value being 0.35 ml and 0.225 respectively as compared to ranitidine (15 mg/kg) where the mean value was 0.5 ml. The value obtained with the combination of ranitidine and all the two doses of NLE were found to be statistically significant as 'p' values was < 0.01(Table-13). The % inhibition is 24.92%, 47.45% and 66.22% with ranitidine 15 mg/kg, ranitidine (15 mg/kg)+NLE(20 mg/kg), and ranitidine (15 mg)+NLE(40 mg/kg) respectively (Fig.- 1).

Further, ranitidine in a dose of 15 mg/kg did not significantly decrease ulcer index in pyloric ligated mice (Table-3) and Fig.-2). Low doses of NLE have also been found to lack significant ulcer protective effect as has been observed in the study. The combination of 15 mg/kg of ranitidine with NLE in doses of 20 and 40 mg/kg was, however found to produce a decline in the ulcer index and the mean ulcers per mice in a

dose dependent manner (Table-3 and 5, Fig.-2 and 3). Significant results were observed with all these doses, the combination of ranitidine (15 mg/kg) with 20 mg/kg and 40 mg/kg of NLE producing highly significant reduction ($p < 0.01$) in all these parameters. The % of inhibition of ulcer index is 14, 66.15 and 95.38% with ranitidine 15 mg/kg, ranitidine (15 mg/kg)+NLE (20 mg/kg), and ranitidine(15 mg/kg)+NLE(40 mg/kg) respectively(Fig.-2).

The percent of inhibition of number of ulcer per mice is 28.91, 49.98 and 55.29% with ranitidine 15 mg/kg, ranitidine (15 mg/kg)+NLE (20 mg/kg), and ranitidine (15 mg/kg)+NLE (40 mg/kg) respectively (Fig.- 3).

From the above result it is evident that ranitidine at a dose of 15 mg/kg does not possess significant ulcer protective property. The same result were observed with NLE at doses of 20 mg/kg and 40 mg/kg. However, when two drugs in the aforementioned doses are combined, significant results are obtained. Thus, it may be concluded that ranitidine and NLE in combination produce synergistic effects.

Anticholinergic action of nimbidin has also been reported by Pillai *et al.* (1978), which they observe, possibly explains its anti-secretory and anti-ulcer genic effect.

Neem leaves aqueous extract inhibits $H^+ K^+$ ATPase activity in vitro in concentration dependent manner to inhibit acid secretion, which may possibly explains its anti-secretory and anti-ulcer genic effect of NLE.

V. CONCLUSION

This study showed that ranitidine at a dose of 15 mg/kg does not possess significant ulcer protective property. The same result were observed with NLE at doses of 20 mg/kg and 40 mg/kg. However, when two drugs in the aforementioned doses are combined, significant results are obtained. Thus, it may be concluded that ranitidine and NLE in combination produce synergistic effects.

5.1 Authors's Statements

Competing interests

The authors declare no conflict of interest.

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