



EFFECT OF NEEM (AZADIRACHTA INDICA) ON PEPTIC ULCER IN ALBINO MICE

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ABSTRACT

The objective of this study was to evaluate the antiulcer effects of Neem on aspirin induced gastric ulceration in albino mice. Ranitidine, the most widely used and common H₂ blocker was taken. Ulcer induction was done by the administration of aspirin orally. This method was chosen because administrations of aspirin result in the production of gastric mucosal damage mainly in the glandular segment of the Albino mice stomach, which is analogous to the body of stomach in man. The freshly prepared aqueous solution of Neem administered in doses of 20, 40, 80, and 160 mg/kg body weight produced dose dependent reduction in ulcer index against aspirin induced ulcers in different groups of mice. The reduction in ulcer index with Neem was statistically significant at 40 and 80 mg/kg body weight and highly significant protection was observed with 160 mg/kg of Neem. Neem in a dose of 160 mg/kg is more effective than ranitidine (25 mg/kg) as regards its antiulcer activity. This study had also established that a low dose of Neem produced equally beneficial effect in reducing gastric ulcer. Thus from the above study it may be inferred that Neem possess a significant and definite ulcer protective action. It is also postulated that the ulcer protective action of Neem is due to inhibition of H⁺ K⁺ ATPase activity in concentration dependent manner. This ultimately leads to conclusion of antiulcer properties of Neem.

Keywords: Aspirin, Gastric, Induction, H₂ Blocker, Ranitidine.

I. INTRODUCTION

Peptic ulcer disease is the most prevalent gastrointestinal disorder. Peptic ulcer disease encompassing gastric and prostaglandin E₂ (PGE₂) content and proinflammatory duodenal ulcer is the most prevalent gastrointestinal cytokines interleukin (IL)-1 and tumor necrosis factor disorder. A peptic ulcer is a defect in the lining of the stomach or the first part of the small intestine, an area called the duodenum. A peptic ulcer in the stomach is called a gastric ulcer. Peptic ulcers are chronic, the most often solitary, lesions that occur in any portion of gastrointestinal tract exposed to the aggressive action of acid – peptic juices. A peptic ulcer, also known as peptic ulcer disease (PUD), is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. It is defined as mucosal erosions equal to or greater than 0.5 cm [1]. Gastric ulcers have also been suggested to occur due to an imbalance between the levels of defensive factors and destructive injurious by products in the gastric mucosa [2, 3].

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases [4]. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors [5]. Peptic ulcer is a sore on the lining of the gastrointestinal tract caused due to mucosal erosions [6]. It can be classified mainly into four types they are gastric, duodenal, esophageal and Meckel's Diverticulum ulcers [7]. Exposure to ulcerogens results in excessive production of reactive oxygen species (ROS) which are harmful for the gastric mucosa [8], whereas the mucus layer and endogenous antioxidants which are part of the gastrointestinal defence help in the protection against ROS induced cytotoxicity [9,10]. Neutrophil infiltration has also been suggested to be a critical component in the development of gastric ulcers. The enzyme myeloperoxidase is used as an indicator of neutrophil infiltration in gastric ulcer pathogenesis [9, 11-14]. The predominant causes of peptic ulcer are infection with the bacterium called *Helicobacter pylori* (*H. pylori*) and the use of Non-steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin and ibuprofen [15]. In India PUD is very common and in the Indian Pharmaceutical Industries, anta-acids and anti-ulcer drugs share 6.2 billion rupees and occupy 4.3% of market share. Peptic ulcers are produced mainly by an imbalance between the gastro duodenal mucosal defense mechanisms and the damaging forces.

A number of plant drugs are also believed to possess anti-ulcer properties, one of these plants is *Azadirachta indica* A.juss commonly known as Neem. Neem is truly a tree with roots firmly embedded in the cultures of its people. For 2000 years in India [16] neem twigs have been chewed on to clean teeth, neem leaf juice applied on skin to treat disorders, neem tea drunk as a tonic, and neem leaves placed in the home to ward away bugs [17]. *A.indica* (Neem), grown in abundance in the Indian subcontinent, has occupied a place of pride in ancient Indian traditional medicine and has universally been accepted as a wonder tree. *Azadirachta indica* or *Margosa* popularly known as Neem is a very common tree and is known in Indo-Pak for as long as 5000 years [18].

Azadirachta indica has many chemical constituents that acts against development of ulcers [19], such as decrease in acid and pepsin secretion [20], proton pump inhibition and antioxidant effects [21], Deglycyrrhizinated licorice extract shows antiulcer, antioxidant activity [22] and healing of ulcers [23, 24] 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 [25] 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 [26].

Neem (*Azadirachta indica*) bark aqueous extract has potent antisecretory and antiulcer effects in animal models and has no significant adverse effect. When compared with the currently used antiulcer drugs such as ranitidine and omeprazole, the crude bark extract was found to be more effective than ranitidine but almost equipotent to omeprazole in its antiulcer effect, whereas in its acid inhibitory effect, it is more or less equipotent to both ranitidine and omeprazole [26]. Preparation of almost all parts of *A.indica* has got curative, preventive and healing effect in a variety of disease conditions. Though widely used in many of disease

condition in India, it mostly remained a neglected topic because of any proper scientific enquiry into its various healing and curative properties.

II. MATERIALS AND METHODS

The Chemicals used are as follows:

2.1) Chemicals:

2.1.1) Carboxymethyl 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 $22 \pm 2^{\circ}\text{C}$ with 12 h light/dark cycle.

Swiss albino mice were 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.

2.4) METHODS

This study will mainly focus upon the healing properties of NLE (Neem leaf extract) in aspirin induced ulcers and pyloric-ligated ulcers in albino mice in order to throw further light on the antiulcer properties of NLE.

Work plan:

The study will be carried out in following parts:-

2.4.1) Preparation of NLE (Neem leaf extract).

2.4.2) a) Induction of ulcer by aspirin

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

2.4.3) 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.)]

2.4.4) Statistical analysis of observed data.

III. RESULTS

EFFECT OF NLE ON ULCER INDEX IN ASPIRIN TREATED MICE

	Aspirin treated	Ranitidine (25 mg/kg)	NLE (20 mg/kg)	NLE (40 mg/kg)	NLE (80 mg/kg)	NLE (160 mg/kg)
Mean	0.575	0.0267	0.423	0.152	0.078	0.0167
SE	0.08932	0.00422	0.02275	0.02136	0.00646	0.00843

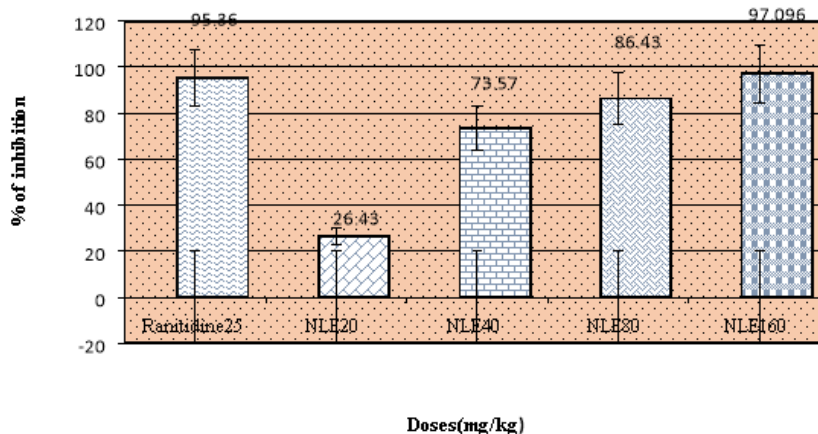
ANALYSIS OF VARIANCE (ANOVA)

Source of Variation	Sum of Square	df	Mean square	F
Between	1.622	5	0.3245	37.19**
Error	0.2617	30	8.7245 E-03	
Total	1.884	35		

df= Degree of freedom

Referring to the table of F, for $p = 0.01$ against 5 df between mean square and 30 df for within mean square, we find a value of 3.7. Since the value 37.19 for F obtained in the present experiment is far greater than the recorded value 3.7. Hence the decrease in ulcer index is significant.

FIG-1: EFFECT OF RANITIDINE AND NLE ON ULCER INDEX IN ASPIRIN TREATED MICE.



EFFECT OF NLE ON ULCER INDEX IN PYLORIC-LIGATED MICE.

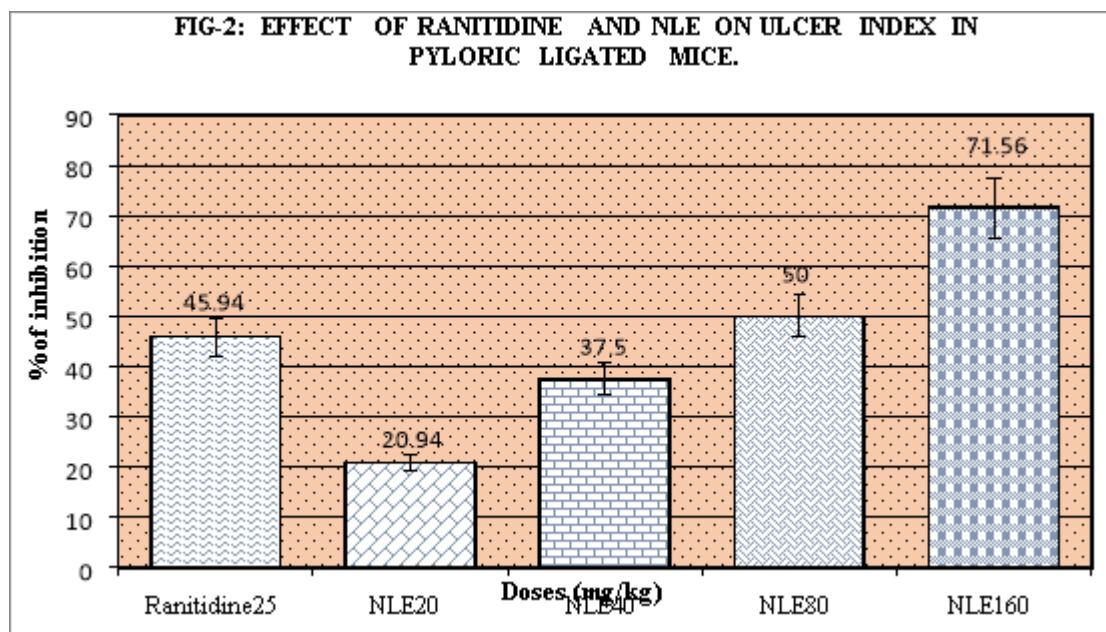
	Control	Ranitidine 25 mg/kg	NLE in mg/kg			
			20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Mean	0.320	0.173	0.253	0.20	0.16	0.09
SE	0.03066	0.01229	0.01116	.01125	0.01291	0.00792

ANALYSIS OF VARIANCE (ANOVA)

Source of Variation	Sum of Square	df	Mean square	F
Between	0.1877	5	3.7549E-02	23.89**
Error	4.7150E-02	30	1.5717E-03	
Total	0.2349	35		

df= Degree of freedom

Referring to the table of F, for $p = 0.01$ against 5 df between mean square and 30 df for within mean square, we find a value of 3.7. Since the value 23.89 for F obtained in the present experiment is far greater than the recorded value 3.7. Hence the decrease in ulcer index is significant.



EFFECT OF RANITIDINE AND NLE ON NUMBER OF ULCERS/MICE IN PYLORIC LIGATED MICE.

	Control	Ranitidine 25 mg/kg	NLE in mg/kg			
			20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Mean	6.666	1.33	4.83	4.50	3.33	0.83
SE	0.61464	0.33333	0.74907	0.76376	0.61464	0.40139

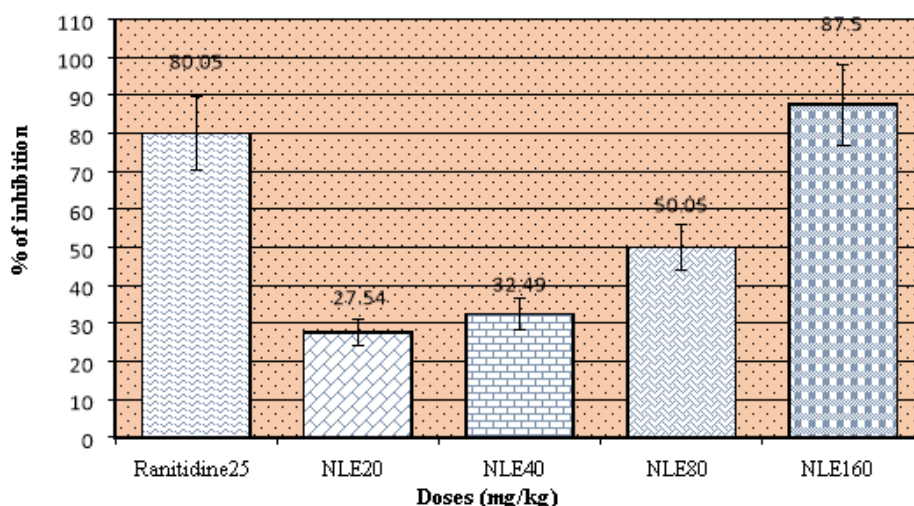
ANALYSIS OF VARIANCE (ANOVA)

Source of Variation	Sum of Square	df	Mean square	F
Between	147.6	5	29.52	13.59**
Error	65.17	30	2.172	
Total	212.7	35		

df= Degree of freedom

Referring to the table of F, for $p = 0.01$ against 5 df between mean square and 30 df for within mean square, we find a value of 3.7. Since the value 13.59 for F obtained in the present experiment is far greater than the recorded value 3.7. Hence the decrease in number of ulcers is significant.

FIG-3 : EFFECT OF RANITIDINE AND NLE ON NUMBER OF ULCER IN PYLORIC LIGATED MICE .



In this study, ulcer induction was done by the administration of aspirin orally. This method was chosen because administrations of aspirin result in the production of gastric mucosal damage mainly in the glandular segment of the Albino mice stomach, which is analogous to the body of stomach in man [27]. The details of the procedure have been described in the section on material and method.

The result on the present study revealed that ranitidine as a standard drug with a dose of 25 mg/kg body weight i.p produced significant reduction in the ulcer index in aspirin-induced ulcers in albino mice. This proved ranitidine to be highly efficacious ulcer protective agent, which is in corroboration with its excellent clinical efficacy. As it is evident that ranitidine of 25 mg/kg is almost equivalent to NLE 160 mg/kg in preventing ulcer index in aspirin treated Albino mice. As it is evident, the freshly prepared aqueous solution of NLE administered in doses of 20, 40, 80, and 160 mg/kg body weight produced dose-dependent reduction in ulcer index against aspirin-induced ulcers indifferent groups of mice. The doses of NLE were selected according to Garg [25], who have studied NLE against stress ulcers in mice.

The reduction in ulcer index with Neem was statistically significant at 40 and 80 mg/kg body weight and highly significant protection was observed with 160 mg/kg of NLE. The result are depicted in the form of bar diagrams, which shows that NLE in a dose of 160 mg/kg is more effective than ranitidine (25 mg/kg) as regards its antiulcer activity. Pillai [24] in their studies on nimbidin (active principal derived from oil of seed and trunk of *A.indica*) observed similar dose-dependent reduction of ulcer index, ulcer score and the mean ulcer per albino mice with significant reduction at a dose 20 mg/kg of nimbidin. Our finding are also in agreement with the Garg [25], who had reported ,dose-dependent ulcer protective action of Neem leaves against stress induced ulcers in albino mice. However, there are wide variation in ulcers indices recorded by

different workers. In our study, the mean ulcer index in the aspirin treated group was 0.575 ± 0.089 . Pillai [24] observed a mean ulcer index of 19.31 in pyloric ligated albino mice and a mean of 20.4 in histamine induced lesion in guinea pig. Garg [25] reported a mean of 4.64 ± 0.8 in stress ulcers in albino mice. This variation could be explained on the basis of different method of calculation of ulcers index adopted by different worker. We have followed the method of Ganguly and Bhatnagar [28] where Pillai [24] have derived the ulcer index as sum of the ulcers incidence divided by 10. Garg [25] have calculated the index by the method of Ogle, Cho and Wong [29]. The above discussion establishes a definite ulcer protective action of NLE in aspirin induced ulcers in albino mice with significant protection being offered at a dose of 40 mg/kg of body weight.

IV. CONCLUSION

This study showed that, NLE was effective in producing a dose dependent reduction in ulcer index and ulcer score in aspirin-treated albino mice. This result was significant when administered, starting with a dose of 40mg/kg of body weight in an increasingly upward mode. Concomitant with these finding, NLE also produced a significant decline in the ulcer index, ulcer score and number of ulcers per albino mice in gastric mucosa. Ranitidine in a dose of 15 mg/kg of body weight did not have any significant in ulcer healing effect or reducing the secretion. But when ranitidine was administered in a dose of 25 mg/kg of body weight, it exhibited significant ulcer protective properties in both model of this study. However, much more work remains to be done such as isolation of various active principal of NLE, further animal studies and a well laid out clinical trial before *A.indica* leaves could be marketed as an effective antiulcer drug.

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