IMPACT OF LEAF EXTRACT OF NEEM(AZADIRACHTA INDICA) ON GASTRIC ACID SECRETION IN ALBINO MICE

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ABSTRACT

The effect of NLE was studied on separate set ulcer model, which enabled detailed observation of the antisecretory and antiulcer effect of NLE. NLE in dose of 20,40, 80 and 160 mg/kg was administered by subcutaneously route immediately after pyloric-ligation. The advantage of this method is that it allows analysis of the gastric contents (volume, pH, pepsin, mucous, polysaccharides etc) and study of the stomach mucosa as well for the occurrence of ulceration, petechiae, hemorrhage etc. Anatomically, the Albino mice stomach is divided into upper two-fifth non-secretory portion and lower three-fifth glandular secretory portion, which resembles the body of the stomach in man both anatomically and functionally. NLE within the dose range of 20 to 160 mg/kg reduced the mean volume in a dose-dependent manner. The range gastric secretion in each group gradually decreased with concomitant increase in dose. A 160 mg/kg dose NLE produced a volume reduction to fifty percent of the control value. The volume of gastric secretion was significantly reduced by ranitidine 25 mg/kg. This experiment has clearly brought in to focus the effect of NLE in reducing gastric volume of the gastric acids content. The optimum result has been obtained in the dose of 80mg/kg and 160mg/kg of body weight. Following the administration of NLE in doses of 20, 40, 80 and 160 mg/kg, the value of mean number of ulcers per albino mice stomach recorded in this investigation were 4.67, 4.3, 3.1 respectively. Significant reduction was obtained by dose of 80 mg/kg and 160 mg/kg in our study.

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INTRODUCTION

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 secrections 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 sulphhydryl 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 proinflammatory 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]. Aspirin is a potent nonsteroidal 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. Impairment of gastric ulcer healing depends upon the augmented release of pro-inflammatory cytokines[14]. Literature review revealed that oxidative stress too plays a pivotal role in progression of ulcer that directly impaired cells functions [15]. ROS plays a vital role in apoptotic process, which involves the release of proteins that triggers the activation of caspases-3, caspases-4 and Cytochrome c. [16,17]. Matrix metalloproteinase (MMPs) an enzyme plays a major role in the ulcer tissue remodeling[18,19]. NO generated from the endothelial nitric oxide synthase (e-NOS) plays an important role in ulcer healing by promoting angiogenesis regulates gastric mucosal blood flow and stimulates gastric mucus secretion[20-22]. Further, Growth factors are too implicated in ulcer healing[23]. It has been shown that various experimental methods are available for the induction of ulcer which includes ethanol-induced ulcers, pylorus ligation-induced ulcers, stressinduced ulcers, acetic acid-induced ulcers and reserpine-induced gastric ulcers[24,25].

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to reports of the World Health Organization, 80% of the world’s population relies mainly on traditional therapies, which involve the use of plant extracts or their active substances (World Health Organization (WHO)) [26]. Flavonoids have anti-inflammatory activity and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species. Plants containing flavonoids were found to be effective in preventing this kind of lesion, mainly because of their antioxidant properties. The antioxidant activity of flavonoids has attracted interest because of the strong evidence that oxidation processes are involved in the mechanisms of several gastric disorders, including ulcerogenesis [27,28].

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. Different parts of the plant have been reported to possess medicinal properties like hypoglycemic, anti septic, wound-healing, curing of skin diseases and anti ulcer activities. Pillai [29] reported the ulcer protective effects of nimbidin, the active principle obtained from Neem seed oil and the bark of Neem tree, in thes histamine-induced lesions in guinea pigs. Garg[30]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 [31].

PLAN OF WORK (MATERIALS AND METHODS)

Materials:

a) Chemicals
The Chemicals used were as follows:

a) Cerboxymethylcellulose

b) Drug
a) Neem leaf extract
b) Ranitidine (Torrent Pharmaceuticals)
c) Aspirin
d) Anaesthetic ether
c) **Experimental Laboratory animals**
Swiss albino mice (*Mus musculus*) will be selected as the experimental animals.
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.

**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 anti ulcer properties of NLE.

**Work plan: **
The study will be carried out in following parts:
Part-1 Preapration of NLE ( Neem leaf extract )
Part-2; a) Induction of ulcer by pyloric-ligation.
    b) calculation of ulcer index.
( ulcer index=10/X  ( where X = total mucosal area/total ulcerated area.)
Part-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 ]
Part-4 ; Statistical analysis of observed data.

**RESULTS**

1 : Effect of NLE on volume(in ml)of gastric secretion in pyloric-ligated mice.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ranitidine 25 mg/kg</th>
<th>NLE in mg/kg 20 mg/kg</th>
<th>NLE in mg/kg 40 mg/kg</th>
<th>NLE in mg/kg 80 mg/kg</th>
<th>NLE in mg/kg 160 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.66667</td>
<td>0.36</td>
<td>0.5</td>
<td>0.413</td>
<td>0.343</td>
<td>0.333</td>
</tr>
<tr>
<td>SE</td>
<td>0.8819</td>
<td>0.06175</td>
<td>0.2633</td>
<td>0.3412</td>
<td>0.4177</td>
<td>0.05578</td>
</tr>
</tbody>
</table>

**Analysis of Variance (ANOVA)**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of square</th>
<th>d.f</th>
<th>Mean square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>0.4963</td>
<td>5</td>
<td>9.9264E-02</td>
<td>5.423</td>
</tr>
<tr>
<td>Error</td>
<td>0.5491</td>
<td>30</td>
<td>1.8304E-02</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.045</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f= Degree of freedom.

Referring to the table of F, for p =0.01 against 5 d.f between mean square and 30 d.f for within mean square , we find a value of 3.7. Since the value 5.423 for F obtained in the present experiment is the far greater than the recorded value 3.7. We conclude that the excision wound contraction area is highly significant (P < 0.001).
Graph:-

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ranitidine (15mg/kg)</th>
<th>Ranitidine(15mg/kg)+ NLE(20mg/kg)</th>
<th>Ranitidine(15mg/kg)+ NLE(40mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.666</td>
<td>0.5</td>
<td>0.35</td>
<td>0.225</td>
</tr>
<tr>
<td>SE</td>
<td>0.8819</td>
<td>0.03651</td>
<td>0.07188</td>
<td>0.04787</td>
</tr>
</tbody>
</table>

Analysis of Variance (ANOVA)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of square</th>
<th>d.f</th>
<th>Mean square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>0.6553</td>
<td>3</td>
<td>0.2184</td>
<td>8.789</td>
</tr>
<tr>
<td>Error</td>
<td>0.4971</td>
<td>20</td>
<td>2.4854E-02</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.152</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f= Degree of freedom.

Referring to the table of F, for p = 0.01 against 3 d.f between mean square and 20 d.f for within mean square , we find a value of 4.94. Since the value 8.789 for F obtained in the present experient is the far greater than the recorded value 4.94. We conclude that the excision wound contraction area is highly significant (P < 0.001).

Graph:-

Fig-2: Effect of Ranitidine and NLE on volume of gastric acid secretion in pyloric ligated mice.
3: Effect of Ranitidine and NLE on number of ulcers/mice in pyloric-ligated mice.

<table>
<thead>
<tr>
<th>Control</th>
<th>Ranitidine 25 mg/kg</th>
<th>NLE in mg/kg 20 mg/kg</th>
<th>NLE in mg/kg 40 mg/kg</th>
<th>NLE in mg/kg 80 mg/kg</th>
<th>NLE in mg/kg 160 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>6.666</td>
<td>1.33</td>
<td>4.83</td>
<td>4.5</td>
<td>3.33</td>
</tr>
<tr>
<td>SE</td>
<td>0.1464</td>
<td>0.3333</td>
<td>0.74907</td>
<td>0.76376</td>
<td>0.61464</td>
</tr>
</tbody>
</table>

Analysis of Variance (ANOVA)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of square</th>
<th>d.f</th>
<th>Mean square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>147.6</td>
<td>5</td>
<td>29.52</td>
<td>13.59</td>
</tr>
<tr>
<td>Error</td>
<td>65.17</td>
<td>30</td>
<td>2.172</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>212.7</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f= Degree of freedom.

Referring to the table of F, for p = 0.01 against 5 d.f between mean square and 30 d.f for within mean square, we find a value of 3.7. Since the value 13.59 for F obtained in the present experiment is the far greater than the recorded value 3.7. We conclude that the excision wound contraction area is highly significant (P < 0.001).

Graph:

The effect of NLE was compared with that of the H2 blocker ranitidine taken as the standard drug. The total volume of the gastric content in the control group was in the range of 2-5 ml with a mean volume of 3.33 ml. Pillai et al. (1978) have reported control volume of 6.019 ml in their study. They had administered vehicle of the test drug orally one hour prior to pyloric ligation. This could be one of the causes of higher volume of gastric content. In this study the vehicle has been administered subcutaneously. NLE within the dose range of 20 to 160 mg/kg reduced the mean volume in a dose-dependent manner. The range gastric secretion in each group gradually decreased with concomitant increase in dose. A 160 mg/kg dose NLE produced a volume reduction by fifty percent of the control value. The volume of gastric secretion was significantly reduced by ranitidine 25 mg/kg. This experiment has clearly brought in to focus the effect of NLE in reducing gastric volume of the gastric acids content. The optimum result has been obtained in the dose of 80 mg/kg and 160 mg/kg of body weight.

As evident from graph ranitidine 25 mg/kg is almost equivalent to NLE 80 and 160 mg/kg in preventing volume of gastric acid secretion in pyloric ligated albino mice. The effect of ranitidine is same as NLE 160 mg/kg in reducing no. of ulcers in pyloric ligated albino mice which is above 80%. In comparison, NLE 80 mg/kg is less effective with % inhibition of 50 as compared to 82 of ranitidine 25.

Following the administration of NLE in doses of 20, 40, 80 and 160 mg/kg, the value of mean ulcer per albino mice stomach recorded in this investigation were 4.67, 4.3, 3.1 respectively. Similar finding had also been reported by Pillai et al. (1978). Both NLE and nimbidine have reduced the number of ulcers per albino mice progressively with increasing doses. Significant reduction was obtained as dose of 80 mg/kg in our study and at 20 mg/kg by Pillai et al. (1978).

In the light of the above discussion, it is evident that NLE possess a definite and significant antiulcer effect in albino mice.
CONCLUSION
This study showed that ranitidine 25mg/kg is almost equivalent to NLE 80 and 160mg/kg in preventing volume of gastric acid secretion in pyloric ligated albino mice. It is clearly indicated that ranitidine 25mg/kg is equivalent to NLE 80mg/kg in prevention of ulcer index in pyloric ligated albino mice. Even better results are seen with NLE 160mg/kg which has % inhibition of 69 as compared to 47 in case of ranitidine.

Authors’s Statements:
Competing interests
The authors declare no conflict of interest.

REFERENCES