Evaluation of antiulcer activity of aerial part extract of *Enhydra fluctuans*: An experimental study

Manoj Kumar Sethi, Laxmidhar Maharana, Snigdha Pattnaik*
School of Pharmaceutical Sciences, Siksha O Anusandhan Deemed to be university, Kalinga Nagar, Bhubaneswar-751003 INDIA

ABSTRACT:

*Enhydra fluctuans* (Family: Asteraceae) a hydrophytic plant known as marsh herb growing throughout the year and adequately available in north east region of India. This plant is used as folk medicine for its diversified medicinal benefits. Current experimentation was performed to scientifically validate the antiulcer potential of the plant extract by using rat ulcer models (pylorus ligation rat ulcer model and ethanol induced ulcer model). Based upon result of acute toxicity study two doses (low dose 200mg/kg and high dose 400mg/kg) of methanol extracts of aerial part of the plant were selected for evaluation. The route of administration of the extract was oral for 7 days. Various biochemical parameters (total and free acidity, protein, pepsin, carbohydrate) were observed along with microscopic evaluation of rat stomach. The result revealed that with higher dose of methanol extract of *Enhydra fluctuans* reduction of gastric ulcer can be achieved and was evident in both the rat ulcer model. The outcome of current study holds up the folkloric claim of the plant for treatment in case of peptic ulcer disease hence suggestive of becoming a very potent pharmacological agent against the disease.

Keywords: *Enhydra fluctuans*; pylorus ligation; Carbohydrate; pepsin
INTRODUCTION:

Ulcer remains an utmost factor of concern associated with gastrointestinal disease and 10 % of overall global population affected by this till date with various dissimilar etiologies[1]. Among divergent factors it is proved that smoking, immoderate stress, chronic alcohol intake, prolong use of non-steroidal anti-inflammatory drugs and infection of Helicobacter pylori bacteria contribute significantly to establish the disease. The characteristic features include abdominal pain and mucosal bleeding resulted from inflammation[2,3]. The ulcer can expand due to improper balance between aggressive (acid, pepsin, bile salts and Helicobacter pylori bacteria) and gastro-protective factors (mucus, bicarbonate and prostaglandins) [4]. While looking into the brief epidemiology it can be revealed that most of the ulcers are duodenal (about 19 out of 20). Peptic ulcer causes approximated 15000 fatalities per year by its consequence [5]. In the Indian pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share [6].

Nowadays the most recent perspective of managing ulcer is inhibiting the over secretion of gastric acid, promoting the protective effect of gastric mucosa, inhibiting apoptosis and enhancing the epithelial cell proliferation for productive healing. The accepted treatment of ulcer includes drug of various categories like proton pump inhibitors, antacids, anticholinergics, cytoprotective agents, histamine receptor antagonists and prostaglandin analogues. Many synthetic compounds in the form of drug currently available in market to control the symptoms of this disease and at the very same time alleviating several undesired effect in prolong use and can able to alter the biochemical mechanisms of body. For that purpose searching of safe drug still continues and herbal medicines are found to be best alternative in case of chronic uses [7]. Nowadays also most of the population across the globe still depends upon herbal drugs for initial treatment option due to its safety, acceptability and compatibility profile [8]. In medicinal plants presence of important secondary metabolites like flavonoids and tannins are the active components showing antiulcer activity [9].

*Enhydra flutuans* an annually growing plant mainly hydrophytic in nature and floats on water epidemic to Bangladesh, China, South East Asia, tropical Africa and North East of India i.e. mainly in state Assam [10,11]. This plant possesses several ethno pharmacological significance such as in the treatment of constipation, used in gastric ulcer, in skin disease, in liver disease, used as supplement food source and in diabetes [12-16]. In spite of having the usage in traditional medicine, there are no scientifically proven
information exits for its antiulcer activity. Thus current examination was aimed to evaluate the antiulcer potential of methanol extract of *Enhydra fluctuans* on rat ulcer models.

**MATERIALS AND METHODS:**

**Collection of Plant materials:**

The whole plant was collected from local ponds of Bhadrakh district (Odisha). Before their use, identification and authentication was done by Dr. Pratap Chandra Panda, Taxonomist of RPRC (Regional Plant Resource Centre) Bhubaneswar, Odisha.

**Preparation of Extracts:**

First the plant materials were washed thoroughly and shade dried. After completion of drying it was grinded by the help of a mechanical grinder to obtain coarse powder. Than extraction was done with methanol by soxhalation method for 3 days and the temperature was perpetuated in between 37- 40ºc to conserve thermos-sensitive constituent of the plant.

**Experimental animals:**

Equal proportion of male & female wistar Albino mice (25-30g of B. wt.) were chosen to conduct acute toxicity study. To evaluate antiulcer activities, healthy & adult rats of wistar strain (150-200g B. wt.) were taken. The selected animals have been housed in acrylic cages in well-known environmental conditions (temp: 20–25ºC; relative humidity: 45-55 % under 12 h light/dark cycle). To acclimate the laboratory condition animals were fed with standard rodent diet for 1 week and water *ad libitum* [17]. The experimentation was conducted after obtaining the ethical clearance from institutional animal ethical committee.

**Toxicity studies:**

Acute toxicity study was carried out on overnight fasted swill albino mice up to a dose level of 4000 mg/kg body wt. The methanol extracts administered orally and the animals were under observation for 3 day for their behavioural and physiological changes. The whole process was performed in adherence to OECD-423 guidelines [18].
Antiulcer activity:

Total animals were divided into 4 groups for the study. Each group contained 6 animals. Group-I considered as control and received distilled water 10 mg/kg as treatment. Group-II considered as standard and received pantoprazole 10 mg/kg. Group-III and Group- IV were test group and received methanol extract of both low (200mg/kg) and high (400mg/kg) dose. All treatments were given by oral route for 7 days. The animals were subjected to pylorus ligation on 7th day followed by sacrificing them to collect gastric juice for secretion study and stomach for ulcer index. All the procedure was done under the influence of anaesthesia by using diethyl ether and chloroform.

Gastric juice was collected after 4 hours of pylorus ligation and subjected to centrifuge to obtain the supernatant. The centrifuge fixed at 4000 rpm for 10 min to get the accurate amount of gastric juice. pH meter was used for the determination of pH and then subjected to analysis of various biochemical parameters.

Ulcer index [19], determination of free acidity and total acidity [20], total carbohydrate estimation [21], protein estimation [22] and estimation of pepsin [23] were done by following standard protocol.

In ethanol induced rat ulcer model grouping and treatments were as alike the pylorus ligation model. On the 7th day 90% of absolute alcohol was administered at a dose of 1 ml to all group for induction of ulcer. After 1 hr of alcohol treatment the animals were anaesthetized and sacrificed for ulcer score determination similarly as done in previous model [24].

Protection against ulcer was determined in percentage by using following formulae.

$$\frac{UI \ control - UI \ treated}{UI \ control} \times 100$$

Statistical analysis:

Values for various parameters were expressed as mean±SD. SPSS ver. 16.0 used for significance analysis. The findings of the experiment were subjected to one way ANOVA at both 95% and 99% confidence interval i.e. p-value less than 0.05 was considered as significant at 95% of confidence interval and less than 0.01 was considered as significant at 99% of confidence interval. Finally when p-value obtained less than 0.001 it was considered as extremely significant data.
RESULTS:

Acute toxicity studies of the methanol extracts showed nil mortality up to 4000mg/kg body wt. for 72 hrs hence considered safe for further use. All the animals were carefully observed and no abnormalities were shown during the course of study.

The findings of pylorus ligation rat ulcer model were depicted in Table 1. According to the result better protection against ulcer was obtained by higher dose (400mg/kg) of methanol extract of *Enhydra fluctuans* than that of low dose (200mg/kg). The percentage protection of higher dose treated group was 74.68% which can be comparable to the percentage protection of pantoprazole treated group was 87.75%. The result also revealed that there was significant restoration (p<0.001) of pH and volume of gastric juice in case of both methanol extract (200mg/kg and 400mg/kg) treated group and pantoprazole (10 mg/kg) treated group while compared with normal animals. Estimated result of free acidity and total acidity were included in table 1 which was evidenced of significant decrease (p<0.001) in those parameters in case of methanol extract and pantoprazole treated groups.

In Table 2 representation of the findings of total carbohydrate, total protein and pepsin were given. Insight this it can be observed that there is significant decrease (p<0.001) in the secretion of pepsin whereas significant increase (p<0.001) in mucin content in treated groups. The test results obtained were in adherence with standard drug treatment.

The study results of methanol extract of *Enhydra fluctuans* at both the doses having significant (p<0.001) protective effect against gastric mucosa in ethanol induced rat ulcer model and given in table 3. Better activity was observed in case of higher dose (400mg/kg) than that of lower dose while comparing with standard pantoprazole. The percentage protection for higher dose was 79.33%, for lower dose was 35.48% and for standard drug was 87.79%.

Histopathological evaluation revealed the protective effect against both pylorus ligation ulcer model (Figure 1) and in ethanol induced ulcer model (Figure 2) in comparison with control group.
DISCUSSION:

The pathogenesis of gastric ulcer in many cases are of unidentified etiologies leads to enhanced complication of this disease. The common believed fact that this is the resultant of disturbed balance in between defensive and offensive factors [25]. Man therapeutically active moieties can be used to retrieve the equilibrium including herbal drug [26, 27]. Current research was performed to evaluate the antiulcer potential of one such agent i.e. *Enhydra fluctuans* pylorus ligation and ethanol induced rat ulcer model.

Toxicity study suggestive of the safety profile of methanol extract of this plant to a large extent of dose i.e. 4000mg/kg and can be used for extended duration. It resembles with the findings of previous study of safety concern of this plant [28].

The experimental results of antiulcer evaluation uncovered the fact that methanol extract of *Enhydra fluctuans* at higher dose i.e. 400 mg/kg having potential cytoprotective effect and deprived secretory action and prevents the gastric injury significantly (p<0.001) due to pylorus ligation when administered orally.

Pylorus ligation ulcers usually associated with gastric ulcers, lesions, perforation and haemorrhage due to the mucosal blood flow reduction and imparted gastric mucosal barrier [29, 30]. Another factor of concern is production of large amount of gastric acid and its exposure to unprotected gastric lumen can leads to ulcer [31, 32]. Methanol extracts of *Enhydra fluctuans* can able to reinstates the gastric volume, pH, free acidity and total acidity significantly to furnish its antiulcer activity. Hence the result of this study inferred that some interference exist between the methanol extract and gastric juice accumulation during digestive process. For the rationale it can be reasonable saying that decreased secretory volume and gastric acidity in methanol extract treated animals could be the resultant of histamine blocking action in the stomach [33]. In the motive of this study it was detected that higher dose (400 mg/kg) of methanol extract is fruitful in controlling ulcers produced by hyper acid secretion.

In rats ethanol can induce ulcer by production of reactive oxygen species (ROS) and accountable for intensified damage to the mucosal membrane [34]. Lipid membrane is demolished by the process of lipid peroxidation [35]. These mucosal injury is the consequences of microvascular injury, enhanced permeation, oedema and vascular lifting [36]. Toxic effect of alcohol can be directly involved in decreased mucus
production by reduction of bicarbonate secretion resulting to the necrotic injury to gastric mucosal layer [37]. The outcome of present research suggested that methanol extract of *Enhydra fluctuans* at a dose of 400 mg/kg can able to exert antiulcer effect against alcohol induced ulcer by acting on above mentioned mechanisms.

Herbal drugs contains various secondary metabolites responsible for cytoprotective action and flavonoids are major constituent for this action [38, 39]. Although the exact mechanism behind the ulcer protection of *Enhydra fluctuans* is unexplored it can be believed that the presence of significant amount of flavonoids might be responsible for the gastric defence either by stabilisation of surface epithelial cells or enhanced production of mucus [40].

**CONCLUSION:**

The current research concluded that methanol extract of *Enhydra fluctuans* at a higher dose (400mg/kg) is responsible forcytoprotection against pylorus ligation and ethanol induced rat ulcer model by decreasing the gastric secretory action. By acute toxicity study the safety profile of the plant extract also established and considered safe at very high dose. This can be the suggestive evidence of validating its antiulcer property. Thus it can be used as a novel source of therapeutic agent against gastric ulceration. More studies needed for isolation and purification of active constituents and to establish the exact mechanism of gastro protective action of the plant.

**ACKNOWLEDGEMENT:**

The authors are contented to the department of Pharmacology of School of Pharmaceutical Sciences, Siksha ‘O’ Anusandhan (SOA) Deemed to be University for rendering adequate facility in the laboratory for the purpose of this research and also to Dr. Manoj Ranjan Nayak for his kind support and cooperation.

**CONFLICT OF INTERESTS:**

The authors declare that there was no conflict of interests exists.
REFERENCES:


Table 1: Efficacy of methanol extract of aerial parts of *Enhydra fluctuans* on various parameters in pyloric ligation induced gastric ulcer.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Negative control</th>
<th>pantoprazole</th>
<th>MEEF 200 mg/kg b.w.</th>
<th>MEEF 400 mg/kg b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index (%)</td>
<td>37.80±1.21</td>
<td>5.01±0.26a</td>
<td>24.94±1.58a</td>
<td>9.51±0.29a</td>
</tr>
<tr>
<td>Protection (%)</td>
<td>-</td>
<td>87.75</td>
<td>32.41</td>
<td>74.68</td>
</tr>
<tr>
<td>pH</td>
<td>2.31±0.11</td>
<td>2.98±0.24a</td>
<td>2.68±0.34c</td>
<td>2.83±0.11a</td>
</tr>
<tr>
<td>Gastric juice volume (ml)</td>
<td>5.83±0.22</td>
<td>2.54±0.13a</td>
<td>6.02±0.47</td>
<td>4.27±0.36a</td>
</tr>
<tr>
<td>Free acidity (mEq/l/100 g b.w.)</td>
<td>98.48±3.01</td>
<td>43.58±2.01a</td>
<td>65.23±2.96a</td>
<td>51.02±1.8a</td>
</tr>
<tr>
<td>Total acidity (mEq/l/100 g b.w.)</td>
<td>145.36±5.14</td>
<td>84.59±3.84a</td>
<td>124.24±5.89a</td>
<td>78.57±3.01a</td>
</tr>
</tbody>
</table>

Data represented in the form of mean ± SD. No. of animals (n) = 6 in each group.

*a*p<0.001, *b*p<0.01, *c*p<0.05 while compared with control group.

Table 2: Efficacy of methanol extract of aerial parts of *Enhydra fluctuans* on biochemical markers against pyloric ligation induced gastric ulcer.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Negative control</th>
<th>pantoprazole</th>
<th>MEEF 200 mg/kg b.w.</th>
<th>MEEF 400 mg/kg b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carbohydrates (µg/ml)</td>
<td>527.59±29.84</td>
<td>603.81±19.14a</td>
<td>567.98±23.58a</td>
<td>691.22±12.14a</td>
</tr>
<tr>
<td>Total protein (µg/ml)</td>
<td>426.48±39.24</td>
<td>408.27±21.49a</td>
<td>428.63±24.68</td>
<td>437.21±13.25a</td>
</tr>
<tr>
<td>Pepsin (µg/ml)</td>
<td>25.11±0.69</td>
<td>13.68±0.59a</td>
<td>18.47±0.93</td>
<td>15.13±0.56a</td>
</tr>
<tr>
<td>Mucin activity</td>
<td>1.23±0.76</td>
<td>1.47±0.89a</td>
<td>1.32±0.95a</td>
<td>1.58±0.91a</td>
</tr>
</tbody>
</table>

Data represented in the form of mean ± SD. No. of animals (n) = 6 in each group.

*a*p<0.001, *b*p<0.01, *c*p<0.05 while compared with control group.

Table 3: Efficacy of methanol extract of aerial parts of *Enhydra fluctuans* on ethanol induced rat ulcer model.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Negative control</th>
<th>pantoprazole</th>
<th>MEEF 200 mg/kg b.w.</th>
<th>MEEF 400 mg/kg b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer index (%)</td>
<td>72.21±7.34</td>
<td>9.24±0.78a</td>
<td>43.32±9.03a</td>
<td>14.41±2.23a</td>
</tr>
<tr>
<td>Protection (%)</td>
<td>-</td>
<td>87.79</td>
<td>35.48</td>
<td>79.33</td>
</tr>
</tbody>
</table>

Data represented in the form of mean ± SD. No. of animals (n) = 6 in each group.

*a*p<0.001, *b*p<0.01, *c*p<0.05 while compared with control group.
Fig. 1. Antiulcer effect of different doses of methanol extract of aerial parts of *Enhydra fluctuans* and pantoprazole (10 mg/kg b.w.) on pylorus ligation-induced rat ulcer model.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Negative control</td>
<td>B</td>
<td>Pantoprazole 10 mg/kg</td>
</tr>
<tr>
<td>C</td>
<td>MEEF 200 mg/kg</td>
<td>D</td>
<td>MEEF 400 mg/kg</td>
</tr>
</tbody>
</table>

Fig. 2. Antiulcer effect of different doses of methanol extract of aerial parts of *Enhydra fluctuans* on ethanol-induced rat ulcer model.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Negative control</td>
<td>B</td>
<td>Pantoprazole 10 mg/kg</td>
</tr>
<tr>
<td>C</td>
<td>MEEF 200 mg/kg</td>
<td>D</td>
<td>MEEF 400 mg/kg</td>
</tr>
</tbody>
</table>