Research Article

Anti-ulcerogenic evaluation of the ethanolic extract of water spinach (*Ipomoea aquatica* Forsk) in aspirin ulcerated rats.

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ABSTRACT

Aim of the study is to evaluate the anti-ulcer efficacy of the *Ipomoea aquatica Forsk (*IAF), is known to possess various therapeutic properties. The reason for the study is that, the known non-steroidal anti-inflammatory drugs (NSAIDs) are full of side effects especially ulceration which is at the top. Gastric ulceration is a cause of economic loss and a source of welfare concern worldwide. There are 350,000 to 500,000 new cases per year and more than one million ulcer-related hospitalizations each year. We found that IAF decreased the incidence of ulcers and also enhanced the healing of ulcers. Ethanolic extract of IAF at a dose of 200 and 400 mg/kg was found to be effective by (68.72%) for 200mg/kg and (62.13%) for 400mg/kg in aspirin (ASP) induced ulcer model and significantly reduced free and total acidity; we observed that anti-ulcer effect of IAF may be due to its cytoprotective effect rather than antisecretory activity. Conclusively, IAF was found to possess potent anti-ulcerogenic as well as ulcer-healing properties and could act as a potent therapeutic agent against peptic ulcer disease.

Key Words*: Ipomoea aquatica Forsk;* Peptic ulcer; Aspirin; Non-steroidal anti-inflammatory drugs

1. INTRODUCTION

Ipomoea aquatica Forsk belongs to the family Convolvulaceae grows wild and is cultivated throughout Southeast Asia and is a widely consumed vegetable in the region. Many of the waters where IAF grows serve as recipients for domestic and other types of waste water. Water spinach is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka[1]. Only a very few scientific studies have been conducted on its medicinal aspects. These include the inhibition of effects on liver diseases[2], constipation[3].IA is considered a tonic the species contains several vitamins, including A, B, C, E, and "U" (S-methyl-methionine), and is used to treat gastric and intestinal disorders[4,5,6].The species also contains aliphatic pyrrolidine amides, carotenoids, hentriacontane, ß-sitosterol and its glycosides, prostaglandin, leukotrine, N-trans- and N-cis feruloyltyramines [7,8,9,10,11,12,13]. It is runner type plant with numerous small flowers[14,15,16,17] .The current study was undertaken to evaluate the anti-ulcer activity IAF ethanolic extract by aspirin induced gastric ulcer, till now no pharmacological evaluation has been done on IAF especially in leaf for its anti-ulcer activity. This prompted us to pursue the activity and was examined for their efficacy and for determination of their possible mechanism of action.

2. MATERIALS AND METHODS.

2.1. Plant material.

The fresh leaf's of IAF was collected from (Tiruvannamalai,Tamilnadu,India) Eastern Ghats of South India during June 2006 and the identity of the species was authenticated by Dr.P.Jayaraman,Director,Plant anatomy research centre,Tambaram,Chennai.The specimen voucher was deposited in the Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai.

2.2. Preparation of the Ethanolic Extract of IAF.

The fresh leaf of IAF was collected and washed with running water. It was shade dried at room temperature and 1 kg of

the dried leaf was made in to coarse powder. The powder was passed through a 60 No mesh sieve. Air dried Powdered drug was macerated with ethanol (90 % v/v) in glass percolator and allowed to stand at room temperature for about 24 hours. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The weight of extract obtained was 18.6 %.

2.3. Phytochemical screening:

The freshly prepared leaf extract of IAF was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's, and Dragendorffs reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's,Fehling's and Benedict's reagent; proteins and amino acids with Millon's,Biuret, and xanthoprotein test.Saponins was tested using hemolysis method; Gum was tested using Molishs reagent and Ruthenium red;Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures[18].

2.4. Animals

Swiss albino rats of either sex, weighing 180–200 g were obtained from animal house of C.L.Baid Metha College of pharmacy, Chennai, Tamil nadu, India. Animals were kept in raised mesh bottom cages to prevent coprophagy .The animals were maintained in colony cages at 25±2 æ%C, relative humidity 50–55% maintained under 12:12 h light and dark cycle. The animals were fed with Standard animal feed (Hindustan Lever Ltd.) and water ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conducted between 10-00 and 17.00 h and were in accordance with the ethical guidelines of the International association for Study of Pain[19].All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).the study was approved by the Institutional Animal Ethical Committee (Ref No: IAEC 12/15-CLBMCP, dated 10-10-2007).

2.5. Acute toxicity studies

Acute toxicity study was performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD)[20].

2.6. Treatment schedule

Swiss albino rats of either sex were divided into four groups, each group consists of six animals. All groups of animals received treatments as shown below along with 200 mg/kg of aspirin once daily for 3 days. Group 1 received 1.0 ml/kg p.o 1% SCMC (MERK,India) as vehicle control; Group 2 received 200 mg/kg, p.o ranitidine(sigma chemicals,Bangalore,India) as standard, Group 3 received 200 mg/kg, p.o ethanolic bark extract of IAF,Group 4 received 400 mg/kg,p.o ethanolic extract of IAF.

2.7. Aspirin induced gastric ulcer in rats

The modified method of Geol et al[21], was used for the production of experimental gastric ulceration, i.e. in rats, by administering aspirin (200 mg/kg) suspended in 1% sodium carboxymethyl cellulose. The aqueous suspension of aspirin was administered with the help of a round tip cannula at 12.00 hrs. Ethanolic extracts of IAF, (200 and 400 mg/kg), were administered orally 3 hrs prior to and after the aspirin treatment. This regimen was continued for 3 consecutive days, Following 36 hrs fasting.4 hrs after the aspirin administration the animals were sacrificed by decapitation. The stomach was opened and the percentage inhibition of ulcer was determined[22]. Mean ulcer score for each animal was expressed as ulcer index. The maximum length of each lesion was determined and the sum of the lengths of all lesions in each stomach was expressed as the ulcer index[23, 24].

The curative ratio (C.R.) was calculated according to the formula:

(Control ulcer index - treated ulcer index)/ (control ulcer index) x 100.

The number of ulcers per stomach was recorded, and the percent of ulcer incidence of each group as compared to the control was calculated[25] , the gastric juice was titrated against 0.01N sodium hydroxide using Topfer's reagent as indicator to find out the free acidity and total acidity[26], as per (Table 1)

2.8. Statistical analysis

All values are expressed as mean ±S.E.M. Data of ulcer index was analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison test and other data was evaluated by one-way ANOVA followed by Dunnett's multiple comparison test using Graph Pad PRISM software. *P*-value <0.05 was considered significant.

3. RESULTS

In the aspirin induced ulcer model, it was observed that the treatment with ethanolic leaf extract of IAF (200 and 400 mg/kg) and Ranitidine (200 mg/kg) significantly reduced the lesion index, the total, free acidity as per (Table 1) and the percentage of ulceration, in comparison with negative control

Table 1

Effect of ethanolic leaf extract of IAF on ulcer index, total acidity, and free acidity aspirin induced gastric ulcer in rats.

Treatment (mg/kg)	ulcer index	Total acidity	Free acidity
Vehicle control (1% SCMC)	48.6±4.1	80±6.2	68±6.3
Ranitidine (200 mg/kg)	14.6*±0.8	20*±0.7	14*±1.2
Ethanolic leaf extract of IAF (200 mg/kg)	15.2*±2.1	22*8±1.5	16*±1.8
Ethanolic leaf extract of IAF (400 mg/kg)	18.4*±3.2	35*±2.6	22*±3.2

Each value is the mean±S.E.M. of six determinations. *P < 0.05. Dunnet test as compared to control.

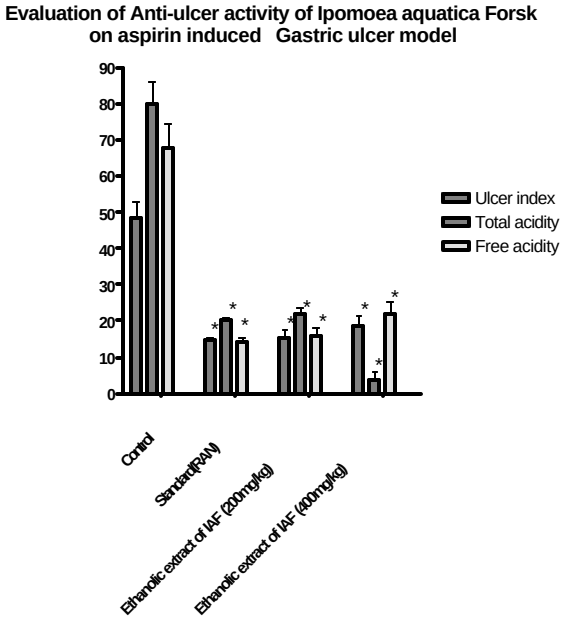
Table 2

Effect of ethanolic extract of IAF against aspirin induced gastric lesion in rats.

S. no.	Treatment Dose (mg/kg)	Mean lesion index	%Ulcer inhibition
1.	Vehicle control 1%SCMC	48.6±4.1	
2.	Ranitidine 200 mg/kg	15.2*±2.1	69.95%
3.	Ethanolic extract of IAF 200mg/kg	18.4*±3.2	68.72%
4.	Ethanolic extract of IAF 400mg/kg	14.6*±0.8	68.31%

Each value is the mean±S.E.M. of six determinations. *P < 0.05. Dunnet test as compared to control.

Fig. 1. Effect of on ulcer index (UI), Free and Total acidity and inhibition of ulcers in Aspirin induced ulcer model. The results are mean±S.E.M. for six rats. Statistical comparison was performed using ANOVA followed by the Dunnet's test. *p < 0.05 in comparison with the control group.



group (p < 0.05). The percentages of inhibition of ulcers were 68.72%, for the test groups with 200 mg/kg of ethanolic leaf extract of IAF and 62.13% for 400mg/kg of ethanolic leaf extract of IAF, when compared to standard group as per (Table 2).

4. DISCUSSION

Peptic ulcer is now believed to be due to an imbalance between the acids and pepsin and defensive factors collectively called the mucosal barrier[27]. Gastric ulcer is usually due to weakening of the gastric mucosa, and duodenal ulcer due to the dominance of acid and pepsin. Risk of ulcerogenesis is now greatly enhanced due to socio-economic problems and exposure of man to many noxious agents and chemicals[28]. Ulcer is the fourth largest disease in Asia. Many drugs available on the market greatly reduce the morbidity and mortality, but may also produce adverse reactions like gynaecomastia[29] and also suffer from high recurrence rates.

Aspirin decreased the concentrations of all the individual carbohydrates and also the carbohydrate to protein ratio;

however a similar decrease in carbohydrate/protein ratio and of individual carbohydrates has been earlier reported in the non-dialysable and lyophilized fractions of the mucus in aspirin-treated dogs [30]. These results tend to confirm that aspirin-like drugs cause ulceration by affecting the mucosal barrier and the carbohydrate/protein ratio of the gastric juice is a good index of the mucus barrier.

The ethanolic extract of IAF at the dose of 200 and 400mg/kg produced a significant decrease in free and total acidity and peptic activity, probably by way of decreasing the glandular secretion. When compare with the negative control (Aspirin) and with that of the ranitidine (standard). In conclusion, it is demonstrated that ethanolic extract of IAF is undoubtedly an anti-ulcer molecule but by multiple mechanisms. The anti-ulcer activity data of IAF signifies that it might be either by increasing the gastric mucosal resistance, local synthesis of cytoprotective prostaglandins synthesis[31]. Hence, it is right to state here that IAF, which is used as a therapeutic agent has an anti-ulcer potential. Further work for its specific anti-ulcer mechanism is in progress.

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