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Research Article

Effect of *Feronia elephantum* (Corr) Fruit Pulp Extract on Indomethacin-induced Gastric Ulcer in Albino Rats

Anurag Mishra*, Sandeep Arora, Rajiv Gupta, Manvi, Rajesh Kumar Punia and Ashish Kumar Sharma

Faculty of Pharmacy, Babu Banarasi Das National Institute of Technology & Management, Sector I, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow, U.P.-227105, India

Abstract

Purpose: *To investigate the activity of Feronia elephantum fruit pulp extract (which is used in folk medicine) against indomethacin-induced gastric ulcer in rats.*
Methods: *The fruit pulp was extracted with ethanol and the anti-ulcer activity of the extract in indomethacin-induced gastric ulceration in Swiss albino rats was evaluated. The parameters assessed were pH and acid concentration of gastric contents, and gastric ulcer index. Ranitidine was used as the reference anti-ulcer drug. Acute toxicity studies were also carried out.*
Results: *The extract (500 mg/kg, p.o.) inhibited indomethacin-induced gastric ulceration by decreasing acid concentration of gastric fluid while elevating its pH ($p < 0.01$), and compared well with the standard drug, ranitidine ($p < 0.001$). However, its anti-ulcer activity was not as potent as that of ranitidine. Acute toxicity studies showed that there was no mortality following the administration of the extract in a dose range of 250 - 5000 mg/kg, p.o..*
Conclusion: *Feronia elephantum fruit pulp extract has potent antiulcer activity with low toxicity. Its anti-ulcer property probably acts via a reduction in gastric acid secretion. The results obtained support the use of this herbal material in folk medicine.*

Keywords*: Anti-ulcer; Feronia elephantum (Corr.); Indomethacin; Ulcer; Gastric acidity*

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Corresponding author:* ***E-mail:* *anupriya0522@yahoo.co.in;* ***Tel: +91-9335288099***

INTRODUCTION

Peptic ulcer disease (PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H_2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects and drug interactions [1]. This is the rationale for the development of new anti-ulcer drugs, and the search for novel molecules has been extended to herbal drugs that would offer better protection and decreased relapse.

Drugs of plant origin are increasing in popularity and are being investigated for a number of disorders, including peptic ulcer [2-3]. *Feronia elephantum* (Corr.) (common names: Bela, Billin, Kath, Kavitha) of the family Rutaceae is native to the Indian subcontinent. The fruit pulp of the plant has been reported in traditional medicine as a curative for various ailments such as diarrheoa, pruritis, impotence, dysentery, heart disease, vomiting, and anorexia, and has also been used for the treatment of asthma and tumours, and as a liver tonic [4]. A decoction (Kadha) administered orally before breakfast has been advocated by local traditional medical practitioners as a tonic purpose [5]. The fruit pulp of *Feronia elephantum* (Corr.) contains flavonoids, phytosterols, tannins, carbohydrates, triterpenoids and amino acids as its chemical constituents [6].

Indomethacin is a potent prostaglandin (PG) biosynthesis inhibitor, and inhibition of prostaglandin synthesis by the drug coincides with the early stages of damage to the cell membranes of mucosal, parietal and endothelial cells. It has been reported that gastric acid secretion is involved in the formation of indomethacin-induced mucosal lesions [7].

The present study was designed to investigate the gastroprotective effect of the ethanol extract of the dried fruit pulp of *Feronia elephantum* on indomethacin-induced gastric ulceration in Swiss Albino rats.

EXPERIMENTAL

Plant material and animals

The plant material was purchased from the local market in Karnataka, India and authenticated by Dr. G.R. Hegde, Professor and head of P.G. Department of Botany, Karnataka University, Karnataka, India and a voucher specimen (no. 01/PG/655/KLE) was deposited in the department's herbarium.

Swiss Albino mice and Albino rats of either sex, each weighing between 20 - 25 g and 150 - 175 g, were selected for acute toxicity and anti-ulcer activity studies, respectively. The experimental method followed was as per the protocol of the institutional Animal Ethics Committee which duly approved the animal studies (ref no. IAEC, Hubli, KLESCOPH/2OO1-02). The animals were acclimatised under standard conditions of temperature (23 ± 1 ^{o}C), relative humidity (55 ± 10 %), 12 h light/12 h dark cycle in the departmental animal house, and given water *ad libitum*. During the period of acclimatisation, they were examined properly for infection and metabolic disorder, and were protected from hurting each other by aggressive behavior. Six animals were kept together as one group.

Extraction

The dried fruit pulp of the plant (250 g) was comminuted to powder passing through a 60 mesh and then extracted with 95 % ethanol using a Soxhlet apparatus. The extract was filtered through cotton wool plug and dried in a rotary evaporator at 40 – 50 ^{0}C under vacuum. Complete dryness was achieved in a calcium chloride desiccator and the dry extract was used for all experimental studies.

Drug and extract standardisation

The extract was suspended in distilled water containing 1% Tween 80 to produce a concentration of 80 mg/ml. Indomethacin I.P. (Micro Labs, Pondicherry, India) was used to induce ulcer in the rats while ranitidine (J.B. Chemical and Pharmaceutical Ltd, Ankaleshwar, India) was used as the standard for anti-ulcer activity.

Acute toxicity studies

The 'Up and Down' or 'Staircase' method was adopted for this evaluation. The extract was administered orally in a dose range of 200 - 5000 mg/kg body weight to ten groups of mice (n = 10). Two mice were orally dosed with 250 mg/kg and observed for a period of 24 h for mortality. In this approach, subsequent doses were then increased by a factor 1.5 if the dose was tolerated, or, decreased by a factor of 0.7 if it was lethal. The maximum non-lethal and minimum lethal doses were determined using 10 mice. Those mice which received doses above 5000mg/kg body weight exhibited ptosis (drooping of upper eyelid) and were observed to be lethargic. Once the approximate $LD_{50,}$ or the range between the maximum non-lethal and minimum lethal dose was found, a final and more reliable LD_{50} assay was performed using at least 3 or 4 dose levels within this range with a larger number of animals in each group. In addition, the source of animal, sex, age, body weight, and presence or absence of any immediate reaction were also recorded as per CPCSEA protocol [8]. The duration of the toxicity test was one week.

Anti-ulcer studies

The maximum non-toxic dose of 5000 mg/kg was obtained from the toxicity studies, and $1/10^{th}$ of this dose (i.e., 500 mg/kg) was taken as the dose for anti-ulcer studies. Albino rats were fasted for a period of 24 h, allowing free access to drinking water *ad libitum* prior to drug administration and divided into four groups of six animals each. Group I was vehicle only control and received normal saline (2 ml/kg). Group II was disease control and received indomethacin in a dose of 20 mg/kg (4 mg/ml dissolved in normal saline containing 0.1% Tween 80). Group III received the extract (500 mg/kg) dispersed in normal saline (80 mg/ml). Group IV received the standard drug, ranitidine (20mg/kg), dissolved in normal saline.

After 30 min, following extract and standard administration, indomethacin was administered intraperitoneally in a dose of 20 mg/kg. Four hours later, under light ether anesthesia, the abdomen was opened by a small middle incision below the xiphiod process, the pyloric portion of the stomach was slightly lifted out and carefully ligated, avoiding traction of pylorus and damage to its blood supply. The stomach was then replaced carefully and the abdominal wall closed with interrupted sutures.
The rats were deprived of both food and water during the post-operative period and sacrificed 19 h after drug administration. The stomach was dissected and its contents drained into a measuring cylinder. The pH of the contents was measured with a digital pH meter (PICO, Labindia Instruments Private Limited).

To determine the concentration of acid, the gastric contents were centrifuged at 1000 rpm for 10 min, 1ml of the supernatant was diluted with 1ml of distilled water, and titrated against 0.1M sodium hydroxide using Topfer's reagent as an indicator until the solution turned orange in colour. Titration was further continued until the solution regained a pink colour. The volume of sodium hydroxide required corresponds to total acidity, expressed as in Eq 1.

Total acidity = volume of sodium hydroxide × normality ×100/0.1 m Eq /l ……. (1)

The stomach was cut open along the greater curvature and the inner surface was examined for ulceration microscopically. Circular lesions indicated ulceration.

Ulceration was quantified by scoring technique whereby normal gastric mucosa was scored 0, punctuate haemorrhage (pinpoint ulcers) was scored 0.5, one or two small hemorrhage ulcer was scored 1.0, and ulcer greater than 3 mm in diameter was scored 2.0. Ulcer index was determined as in Eq 2 [9-11].

Ulcer index = mean degree of ulceration × % group of ulceration/100 …….. (2).

Statistical analysis

The results were expressed as mean ± SEM and statistical significance was evaluated by one-way ANOVA followed by Newman-Keuls Multiple Comparison Test [12]. Significance of difference was accepted as $P < 0.05$.

RESULTS

pH of gastric contents

pH data are shown in Table 1. In control animals, mean pH was 2.90 ± 0.38 and for the indomethacin-treated animals, it was 2.30 ± 0.59. The difference was not significant ($P < 0.01$). However, when either the extract or ranitidine (reference) was administered to the indomethacin-treated group, there was a significant increase in the pH of gastric contents to 3.36 ± 0.09 and 3.46 ± 0.13, respectively ($P < 0.01$).

Acidity of gastric contents

Total gastric acidity, shown also in Table 1, increased insignificantly ($P < 0.01$) following treatment of the rats with indomethacin (0.18 ± 0.03 meq/L) compared to the control (0.14± 0.01 meq/L). On the other hand, administration of the extract significantly decreased gastric acidity (induced by indomethacin). However, extract was more effective than ranitidine in decreasing gastric acidity.

Gastric ulcer index

As indicated in Table 1, administration of indomethacin resulted in the production of gastric lesions. The mean gastric ulcer index for this group (group II) was 5.83 ± 0.87 which is significantly higher ($P < 0.001$) than for the control (0.60 ± 0.42). The extract significantly lowered ($P < 0.01$) the index for indomethacin-induced ulcer to 1.83 ± 0.31. However, ranitidine (with an ulcer index of 0.66.33) showed a more potent anti-ulcer activity ($P < 0.001$) than the extract.

Table 1: Effect of the extract of the fruit pulp of *F. elephantum* (Corr.) on gastric acidity parameters and ulcer index

Group	Treatment	Dose (ml/kg)	pH of gastric contents	Concentration of acid in gastric contents (meq/L)	Gastric ulcer index
I (Normal control)	Control (normal saline)	2	2.90± 0.38	0.14±0.01	0.60±0.42
II (Control)	Indomethacin	20	2.30±0.03	0.18±0.03	5.83±0.87
III	Indomethacin + extract	500	3.36±0.09**	0.08±0.01**	1.83±0.31***
IV (Reference)	Indomethacin + ranitidine	20	3.46±0.13**	0.12±0.01	0.66±0.33***

*Values are expressed as mean±SEM (n = 6); Statistically significant *P<0.05, **P<0.01, ***P<0.001. Data were analysed using one-way ANOVA followed by Newman-Keuls Multiple Comparison Test.*

DISCUSSION

The family, Rutaceae, consists of more than 900 species distributed worldwide. This family contributes a number of medicinal remedies used in the Indian traditional system of medicine. For example, *Aegle marmelos, Citrus medica, Limonea crenulata,* etc, are known to possess a number of therapeutic properties [13]. The genus, Feronia, also belongs to Rutaceae family. The literature on the phytochemical and pharmacological properties of the plants belonging to this family is exhaustive [14]. Surprisingly, however, a literature survey on *Feronia elephantum* (Corr.) revealed that a similar characterisation of this family is less exhaustive. Furthermore, no pharmacological work on its fruit pulp was found in the literature.

Gastric ulcers are due to imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandins [15]. The adrenergic system is involved in gastric secretion [16]. It has been shown that in the gastrointestinal tract, activation of presynaptic α_{2-} adrenoceptors located on the vagus nerve inhibits gastric acid secretion [17].

The plants belonging to the family Rutaceae are known to possess various classes of phytoconstituents including coumarins, flavonoids, essential oil and tannins. As per the acute toxicity data, no mortality of animals was observed. Thus, the ethanol extract of the fruit pulp of *F. elephantum* (Corr.) appears to be safe and non-lethal at a dose as high as 5000 mg/kg. The extract, at a dose of 500 mg/kg body weight, also showed statistically significant anti-ulcer activity but it was not as potent as that of the standard drug, ranitidine. Probably, the flavonoids/ coumarins are the active constituents in the fruit pulp responsible for its pharmacological actions [18]. Although, this was not investigated in the current study, there is a possibility that the anti-ulcer effect of the fruit pulp extract may be due to its effect on various mucosal defensive factors such as prostaglandin accumulation, bicarbonate balance, mucosal glycoprotein, phospholipid layer integrity, tight junctions, cell restitution, cell proliferation and mucosal blood flow [19,20]. Further studies, however, are recommended in order to elucidate fully the chemical and pharmacological properties of the plant material.

CONCLUSION

This study shows the anti-ulcer activity of ethanol extract of the fruit pulp of *Feronia elephantum* in an indomethacin-induced gastric ulcer model. The anti-ulcer property of the extract is probably due to a reduction in gastric acid secretion since it caused an elevation of gastric pH. The results support the traditional use of the plant material in Indian folk medicine.

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REFERENCES

1 Cullen DJ, Hawkey GM, Greenwood *DC. Peptic ulcer bleeding in the elderly: relative roles of Helicobacter pylori and non-steroidal anti-inflammatory drugs. Gut, 1997; 41: 459–62.*

2 *Hoogerwerf WA, Pasricha PJ. Agents used for control of gastric acidity and treatment of peptic ulcers and gastroesophageal reflux disease. In: Hardman JG, Limbird LE, Gilman AG (eds). The Pharmacological Basis of Therapeutics, ed. 10, New York, USA: Goodman and Gilman, 2001, pp 1005-1019.*

3 *Manonmani S, Viswanathan VP, Subramanian S, Govindaswamy S. Biochemical studies on the anti-ulcerogenic activity of Cauvery 100, an ayurvedic formulation in experimental ulcers. Indian J Pharmacol, 1995; 27: 101-105.*

4 *Pandey G. Dravyaguna Vijnana, Varanasi, India, Krishnadas Academy, 2001, p 15.*

5 Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants. A compendium of 500 species. Andhra Pradesh, India, Orient Longman, 1994; pp 327.

6 Kritikar KR, Basu BD. Indian Medicinal Plants. Dehradun, India, Industrial Books Distributors and Publishers, 1995, pp 496-490.

7 Ghosh MN. Fundamentals of Experimental Pharmacology, Calcutta, India, Scientific Book Agency, 1984, pp 156-157.

8 Kulkarni SK. Handbook of Experimental Pharmacology. New Delhi, India, Vallabh Prakashan, 1999, p 75.

9 Gupta M, Mazumder UK, Manikandan L, Bhattacharya S, Senthilkumar GP, Suresh R. Anti-ulcer activity of ethanol extract of Terminalia pallida Brandis in swiss albino rats. J Ethnopharmacol, 2005; 97: 405-408.

10 Kumar A, Rama II. Antiulcer properties of methanolic extract of Benincasa hispida (Thunb.) Cogn. Indian Drugs, 2002; 39: 9-13.

11 Elegbe RA, Bamgbose SA. Protective dietary factors in experimental study of some Nigerian cereals in tubers. Postgrd Med J, 1976; 52: 258-263.

12 Bennett, CA, Franklin NL. Statistical Analysis in Chemistry and Chemical Industry, New York, USA, John Wiley & Sons, 1967, p 133.

13 Sutaria RN. Text book of Systemic Botany. Ahmadabad, India, Khadayata Book Depot, 1969, pp 120-128.

14 David LD, Michael VP, Cohen P. Distribution of Limonoids in the Rutaceae. Phytochemistry, 1972; 11: 705-713.

15 Yamaguchi I, Kumada S. Central and peripheral adrenergic mechanism regulate gastric secretion in the rat, J pharmacol Exp Ther, 1977; 203: 125-131.

16 Jennewein HM. The effect of clonidine on gastric acid secretion in rats and dogs. Naunyn Schmiedebergs Arch. Pharmacol, 1977; 297: 85-90.

17 Dijoseph JF, Taylor JA, Nabimir G. Alpha-2 receptor in the gastrointestinal system: a new therapeutic approach. Life Sci, 1984; 35: 1031-1042.

18 Parmar NS, Parmar S. Antiulcer potential of flavonoids. Indian J Physiol Pharmacol, 1998; 42: 343-51.

19 Goyal RK, Bhattacharya SK. Gastrointestinal mucosal defence and mucosal protective agents, Ind J Exp Biol, 1999; 29: 701.

20 Hawkey, CJ, Rantim, DS. Prostaglandin and gastrointestinal mucosa. Are they important in this function disease or treatment? Gastroenter, 1985; 89: 1162-1165.