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HEALING PROPERTY OF *FICUS EXASPERATA* IN INDOMETHACIN-INDUCED GASTRIC ULCER

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ABSTRACT

The *Ficus Exasperata* (FE) leaves extract was able to promote healing of indomethacin-induced gastric ulcers in mice. In this work we evaluated the antiulcer activity of the leaf extract of FE. The extraction of leaves powder was done in aqueous and methanol solvent. Antiulcer activity of extract was tested in albino rats. Ulceration was induced by indomethacin (30 mg/kg b.w.) through oral route. Esomeprazole was used as a reference drug with a dose of 20 mg/kg b.w., and methanolic FE extract at a dose of 200 mg/kg b.w. once daily four hours after indomethacin administration and lasted for 3 weeks. Ulcer index and % Protective index parameters were thereafter evaluated. The results showed that at 200 mg/kg dose FE showed significant antiulcer activity with ulcer index 6.63 ±0.20 and Protective index 65.88%. Gastric volume also reduced in animal group administered with FE, from 8.28 ± 0.13 to 5.26 ± 0.09. Histopathology study also performed with FE extract. Treatment with FE extract and Esomeprazole for 3 days reduced the number of inflammatory cells and mucosal congestion, and increased the number of healthy normal cells in the gastric mucosa, submucosa, serosa, and muscle layers.

KEYWORDS: *Ficus Exasperata*, Ulcer, Esomeprazole, extraction.

1. INTRODUCTION

Inflammation is elicited in response to harmful stimuli, such as pathogens, mechanical injuries, burns, irritants, and other noxious stimuli that may threaten the well-being of the host. It is marked by local response to cellular injury that is associated with capillary dilatation, leucocytes infiltration, redness, heat, pain, swelling, and often loss of function that serves to initiate mechanisms of eliminating the noxious agents and damaged tissues. It involves a complex array of enzyme activation, inflammatory mediators, fluid extravasation, cell migration, tissue breakdown, and repair.[1]

Gastric ulceration is a benign lesion on the mucosal epithelium on exposure of the stomach to excess acid and aggressive pepsin activity.[2] It is the most prevalent gastrointestinal disorder ever known, accounting for an estimated 15000 mortality yearly.[3] In spite of the rapidly changing concept of gastric ulcer management from conventional vagotomy, prostaglandin analogues, H2 receptor antagonists, and antacids to proton pump inhibitors, gastrointestinal toxicity remains an impediment to their application in clinical practice. Specifically, gastrointestinal toxicity of non-steroidal anti-inflammatory drugs (NSAIDs) origin may be as high as 4–8% per year and the complications are even higher for those with additional risk factors such as prior history of ulcer disease.[4] Investigation on the phytotherapy of medicinal plants that are highly valued and widely used in the traditional systems of medicine might provide efficient formulation for better management.

Ficus Exasperate (FE) is commonly known as ‘‘sand paper tree’’. Phytochemical analysis of the leaf extract of FE has revealed the presence of flavonoids, tannins, saponins, alkaloids, and cyanogenic glycosides.[5] Its medicinal efficacy in treating many diseases has been researched. Its leaf extract is taken to suppress stomach ache, treat peptic ulcer, and as an antidote to poison.[6] With the remarkable attributes of FE particularly in alleviating stomach ache-related disorders and wound healing enhancement, the present study compared their therapeutic efficacy on indomethacin-induced gastric ulceration.

2. MATERIALS AND METHODS

2.1. Plant material

Ficus Exasperata Plant was collected from Mandla Madhya Pradesh. Authentication of plant will do from botany department of Dr. Harisingh Gour University, Sagar M.P. A herbarium was maintained in the Adina Institute of Pharmacy, Sagar. After washing plant all parts was separated (leaves, stem, root, branches, seed, and buds). Leave was used for extraction.

2.2. Extraction of *Ficus Exasperata* leaves

Leaves of FE were chopped into small pieces, air-dried at room temperature for 8 days to a constant weight and subsequently pulverized into fine powder used for the study. The powdered samples (400 g) of each plant were separately suspended in 4 liters of distilled water for 48 hrs. The obtained solution was filtered and the resulting filtrate lyophilized. The resultant solution stored in desiccators for further use.

2.3. Antiulcer activity

2.3.1. Ulcer induction

Gastric ulceration was induced in the animals according to the procedure described by Sayanti *et al.,* (2007).[7] Briefly, rats were administered with a single oral dose of indomethacin (30 mg/kg b.w.). They were deprived of food but had free access to water 24 h prior to ulcer induction. Various degrees of ulceration have manifested 4 h after indomethacin administration.

2.3.2. Animal grouping and treatments

Thirty-five albino rats were randomized into five groups of seven rats each. Group 1 (normal control) animals receive only distill water. Group 2 (ulcerated control) rats received only indomethacin and were sacrificed 4 hours after indomethacin administration. Animals in group 3 were given indomethacin and esomeprazole (20 mg/kg b.w.). Groups 4, 5 and 6 comprised ulcerated rats treated with FE extract (200 mg/kg b.w.). Treatments with the reference drug and extracts commenced four hours after indomethacin administration and lasted for 3 weeks. These were orally administered once daily using oral incubator with ad libitum provision of food and water throughout the experimental period. The protocol conforms to the guidelines of the National Institute of Health (NIH, 1985) for laboratory animal care and use, and in accordance with the principles of good laboratory procedure (WHO, 1998).

2.3.3. Isolation of stomach and collection of gastric juice

On the twenty second day, the animals were humanely sacrificed under diethyl ether anaesthetization. The abdomen was opened and the stomach excised. The stomach was thereafter opened along greater curvature and gastric content was drained into a centrifuge tube. Five ml of distilled water was added and the resultant solution was centrifuged at 3,000 rpm for 10 minutes. The supernatant obtained was thereafter used for biochemical analyses.

2.3.4. Determination of gastric ulceration parameters

Gastric acid output (volume) was determined in the supernatant by titration with 0.0025N NaOH. Free and total acidity were subsequently determined adopting the method of Grossman. The pH of gastric juice was determined using a pH meter, while the procedures of Sanyal *et al.,* (1971) were used to determine specific pepsin activity and mucin concentration respectively.[8]

2.3.5. Quantification of ulceration

Degrees of ulceration in the animals were quantified using the procedure outlined by Tamawski *et al.* (2001).[9] Briefly, cleaned stomachs were pinned on a corkboard and ulcers were scored using dissecting microscope with square-grid eyepiece based on grading on a 0–5 scale (depicting severity of hyperemia and lesions) as follows.

0—almost normal mucosa; 1—hyperemia; 2—one or two lesions; 3—severe lesions; 4—very severe lesions; 5—mucosa full of lesions

*Hyperemia: vascular congestions, Lesions: hemorrhagic erosions.

Areas of mucosal damage were expressed as a percentage of the total surface area of the glandular stomach estimated in square millimeters. Mean ulcer score for each animal was expressed as ulcer index (U.I) and the percentage of ulcer protective index was determined using the expressions.

U.I = [Ulcerated Area/Total stomach area] X 100

% Protective index = [U.I in control- U.I in test] x 100/ U.I in control.

3. RESULT AND DISCUSSION

3.1. Preliminary Phytochemical analysis

Methanol and aqueous *Ficus Exasperata* leaves extracts contained: phenol, tannins, steroids, flavonoids, saponins, alkaloids and glycosides which have been identified by other researchers in various plants and in different parts of plants[10, 11] but the activities of phenol, tannin alkaloids, saponin and glycosides were more pronounced with the methanolic extract as seen in **Table 1.** Saponins are group of steroid or triterpenoid glycosides, also found to be effective antimicrobial substances *in vitro* against a wide array of microorganisms by inhibiting the membrane bound enzymes.[12] They have been reported to possess substantial anti-carcinogenic activities due to their anti-oxidant and inflammatory properties.[13]

Table 1: Phytochemical analysis of *Ficus exasperata* leaves.

Phytochemical	Methanolic Extract	Aqueous extract
Phenol	+	+
Tannin	+	+
Alkaloids		
Wagner's reagent	**+**	**+**
Mayer's reagent	**-**	**-**
Saponin	+	++
Flavanoids	-	+
Terpenoids	-	-
Steroids	+	-
Cardiac glyceride	-	-
Glycoside	++	-

-, not detected; +, present in low concentration; ++, present in high concentration

3.2. Physicochemical evaluation

The physicochemical parameters such as loss on drying (LOD), ash value (Total ash; Acid-insoluble ash; Water-soluble ash, and Sulphated ash) crude fiber, extractive value for new-fangled stem buds of FE powder was performed and results are mentioned in (**Table 2**). The percentage yields of extractive of plants in aqueous and methanol fraction was 1.60 & 6.50%. The aqueous extract showed yellowish green-colored oily substance and methanol extracts showed yellowish brown colored extract soluble in water.

Table 2: Quantitative standards for powder of *Ficus Exasperate* (Values in % w/w with reference to the air-dried drug).

S. No.	Parameters	*Ficus Exasperate*
1.	Loss on Drying	11.20%
2.	Ash Value	
	Total ash	13.50%
	Acid-insoluble ash	2.45%
	Water-soluble ash	7.65%
	Sulphated ash	2.56%
3.	Extractive value	
	Aqueous	1.60%
	Methanol	6.50%

3.3. TLC fingerprint

The TLC plates point out that the phytoconstituents present in the methanol extract of FE was clearly separated chemical moieties (**Figure 1**). There are similar phytocomponents visualized in plant with RF values 0.26, 0.42, 0.65, and 0.85. The methanol extract of plant contains chemical moieties with an equal RF value (0.65) to vallinic acid indicating the presence of a phenolic compound.

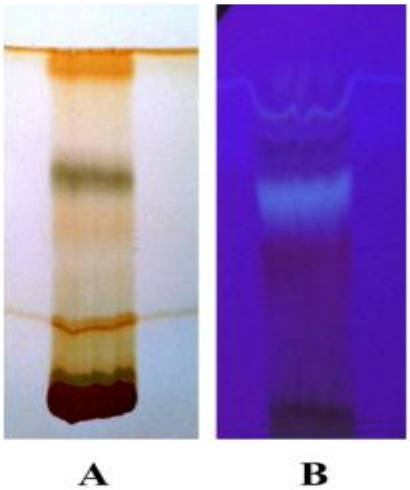

Figure 1: TLC plate A at λ254 nm and plate B at λ365 nm, methanolic extract of *Ficus exasperate*.

3.4. Antiulcer activity

Table 3 showed the effects of leaf extracts of FE on the ulcer index and % ulcer protective index in the experimental animals. Oral administration of 30 mg/kg b.w. of indomethacin caused a significant ($p < 0.05$) increase in the degree of ulceration (Ulcer index) in the rats. A significant improvement in the level of protection against ulceration was however observed following treatment with the extracts.

Table 3: Effect of F. exasperata leaf extracts on ulcer indices of indomethacin ulcerated rats (n = 5, X ± SEM)

Group	Treatments	Ulcer index	% Protective index
1	Distilled water (Normal control)	00.00	-
2	IND (Ulcerated control)	19.14 ± 0.30^{a}	-
3	IND + ESP (20 mg/kg b.w)	3.53 ± 0.12^{b}	83.65
4	IND + F.E	6.63 ± 0.20^{c}	65.88

Values with different superscripts along the same column for the parameters are significantly different ($P < 0.05$).
IND: indomethacin (30 mg/kg b.w.), ESP: esomeprazole (20 mg/kg b.w.), F.E: *F. exasperata* (200 mg/kg b.w.)

Effect of leaf extracts of FE on gastric secretions of indomethacin ulcerated rats was presented in **Table 4**. Indomethacin administration caused significant ($p < 0.05$) decrease in pH value with a corresponding significant ($p < 0.05$) increase in gastric volume and thus, free and total acidity of gastric content. Treatments with the extract produced significant increase in pH value coupled with significant decrease in gastric volume free and total acidity when compared to untreated-ulcerated rats.

Table 4: Effects of leaf extracts of *F. exasperata* on some gastric secretion indices of indomethacin ulcerated rats (n = 5, X ± SEM)

Treatment	Gastric volume	Free acidity	Total acidity	pH
DW (Normal Control)	1.96 ± 0.11^{a}	13.28 ± 0.17^{a}	22.32 ± 0.89^{a}	6.40 ± 0.18^{a}
IND ulcer control	8.28 ± 0.13^{b}	51.90 ± 0.40^{b}	82.84 ± 1.58^{b}	2.30 ± 0.08^{b}
IND + ESP	2.12 ± 0.15^{a}	17.17 ± 0.75^{a}	26.32 ± 0.53^{a}	5.50 ± 0.31^{a}
IND + FE	5.26 ± 0.09^{c}	37.08 ± 0.31^{c}	56.92 ± 0.77^{c}	4.18 ± 0.21^{a}

Values with different superscripts along the same column for the parameters are significantly different ($P < 0.05$).
IND: indomethacin (30 mg/kg b.w.), ESP: esomeprazole (20 mg/kg b.w.), F.E: *F. exasperata* (200 mg/kg b.w.).

Indomethacin administration brought about a significant ($p < 0.05$) increase in specific activity of pepsin as well as significant reduction ($p < 0.05$) in mucin content of gastric juice of ulcerated rats when compared with the normal control (**Table 5**). The observed changes in these parameters were significantly attenuated ($p < 0.05$) after treatment with *F. exasperata*. **Figures 2, 3** revealed the effects of *F. exasperata* on the lipid peroxidation and antioxidant status of gastric mucosal of indomethacin ulcerated rats. MDA level was significantly increased ($p < 0.05$) in the ulcerated animals (**Figure 2**). Interestingly, both extracts in all formulations significantly reduced ($p < 0.05$) the level of MDA comparable to normal. A significant elevation ($p< 0.05$) was also observed in the activities of GSH level (**Figure 3**) in the extracts treated rats. Except for CAT activity, leaves extract of *F. exasperata* remarkably proved effective in ameliorating the effect of indomethacin on stomach antioxidant status of the animals, treatment.

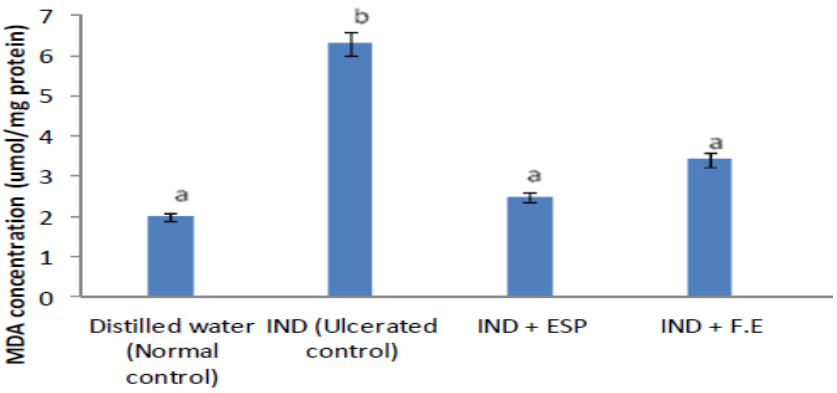

Figure 2: Effect of leaf extracts of *F. exasperata* on gastric malondialdehyde (MDA) level of indomethacin ulcerated rats (n = 5, *X* ± SEM)

Bars with different superscripts for the parameter are significantly different ($P < 0.05$).

IND: indomethacin (30 mg/kg b.w.), ESP: esomeprazole (20 mg/kg b.w.), F.E: *F. exasperata* (200 mg/kg b.w.).

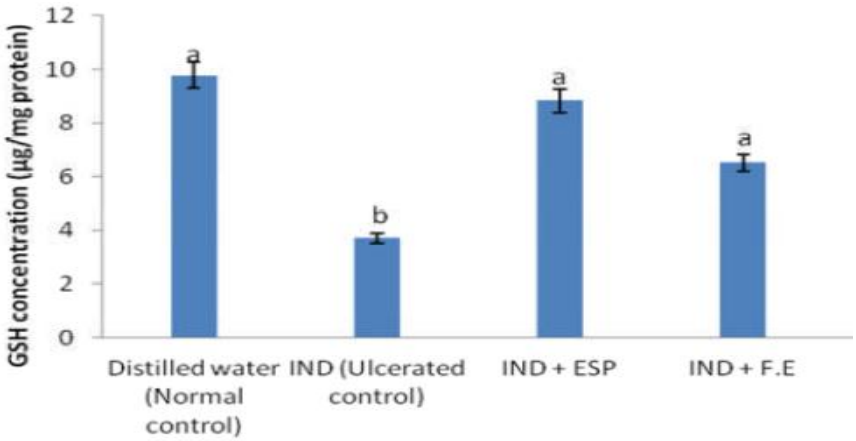

Figure 3: Effect of leaf extracts of *F. exasperata* on gastric reduced glutathione (GSH) level of indomethacin ulcerated rats (n = 5, *X* ± SEM)

Table 5: Effect of leaf extracts of *S. mombin* and *F. exasperata* on gastric pepsin activity and mucin content of indomethacin ulcerated rats (n = 5, X ±SEM)

Group	Treatments	Pepsin activity (µg/ml)	Mucin content (µg/m)
1	Distilled water (Normal control)	100.21 ± 0.03a	396.23 ± 0.20a
2	IND (Ulcerated control)	295.03 ± 0.05b	195.35 ± 0.30b
3	IND + ESP	110.65 ± 0.01a	382.43 ± 0.10a
4	IND + F.E	136.32 ± 0.20a	263.12 ± 0.30c

Values with different superscripts along the same column for the parameters are significantly different ($P < 0.05$). IND: indomethacin (30 mg/kg b.w.), ESP: esomeprazole (20 mg/kg b.w.), F.E: *F. exasperata* (200 mg/kg b.w.).

3.5. Histological assessment

Onset of ulceration (i.e. superficial erosion and mild inflammation in the stomach) was observed within 6 h after indomethacin administration, indicating acute ulceration (data not shown). However, on the third day, marked damage to the glandular portion of the gastric mucosa was noticed in the histological photograph of the stomach sections of the third day-ulcerated group of mice. Hemorrhagic serosa was evident on the third day of ulceration. Treatment with *F. Exasperate* extract and esomeprazole for 3 days reduced the number of inflammatory cells and mucosal congestion, and increased the number of healthy normal cells in the gastric mucosa, submucosa, serosa, and muscle layers. The healing effect of *F. Exasperate* extract was slightly better than esomeprazole. The histological photographs of stomach sections of the third day groups of normal, ulcerated, and treated mice are shown in **Figure 4**.

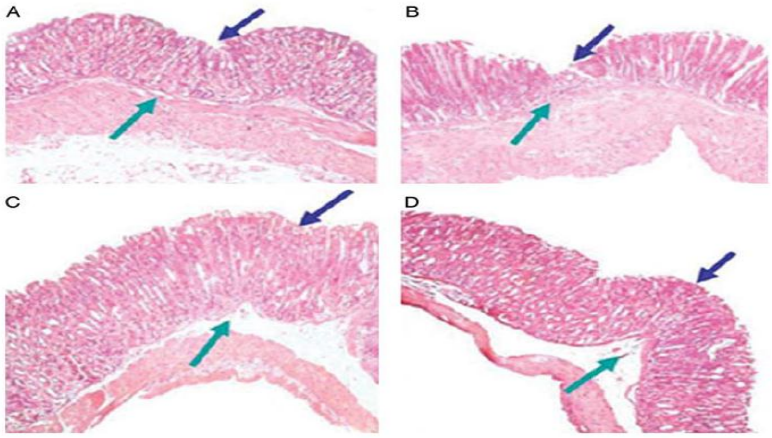

Figure 4: Histological assessment of acute gastric mucosal injury induced by indomethacin in mice and its prevention by *F. Exasperate* extract and esomeprazole Ulceration in the mice was induced by indomethacin (18 mg/kg, single dose, orally). Treatment was carried out with *F. Exasperate* extract (7 mg/kg once daily for 3 days, orally) and esomeprazole (3 mg/kg once daily for 3 days, orally). Representative gastric tissue sections are shown at X 10 magnification. Mucosal and submucosal layers are shown by blue and green arrows, respectively. (A) Sham treated. (B) Ulcerated untreated. (C) Ulcerated + *F. Exasperate* extract treated. (D) Ulcerated + esomeprazole treated.

4. CONCLUSION

The pharmacognostic studies are the first step towards ascertaining the identity and the degree of purity of herbal materials. The pharmacognostic analysis is not reported previously in this plant parts which makes this first report providing complete pharmacognostic profile of leaves of FE and hence will be useful for correct identification and authentication of these plants for future studies. The data collected while performing the morphological, microscopical, physicochemical, and TLC fingerprint analysis mentioned above is useful in the standardization of the selected plant materials. The phytochemical study, evaluation of physical and chemical properties, fluorescence analysis, and phenolic content are very peculiar objectives for the identification of crude drug. Overall, the FE extract was able to promote healing of indomethacin-induced gastric ulcers in mice. This result is corroborated by comparison of its effect with that of the positive control Esomeprazol.

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