ORIGINAL ARTICLE

Comparative and combined studies on anti-ulcer effect of two plant extracts in experimental models of gastric ulcer in SD rats

Gangireddy Ramana, Siva Reddy Challa, Ch. V. Rao[1]

Department of Pharmacology, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, [1]Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Rana Pratap Marg, Lucknow, India

Objective: The present study was designed to carry out comparative and combined effect anti-ulcer studies of *Ficus racemosa* Linn. (Family: Moraceae) (Gular) fruit extract and *Aegle marmelos* (Linn.) Corr. (family: Rutaceae) root extract in various experimental models of ulcer. **Materials and Methods:** Gastro-protective studies were carried out with individual treatments of *Ficus racemosa* fruit extract (FRFE) and *Aegle marmelos* root extract (AMRE) and combination of both in four standard experimental models of ulcer like pylorus ligation (PL)-induced ulcers, Aspirin (ASP)-induced ulcers, Cold-restraint stress (CRS)-induced ulcers, and Ethanol (EtOH)-induced ulcers in SD rats. Gastric secretion parameters, gastric mucosal parameters and anti-oxidant parameters were estimated. **Results:** Both FRFE and AMRE have demonstrated the efficacy in different models of ulcer in the following order: PL-induced gastric ulcers > ASP-induced gastric ulcers > EtOH-induced gastric ulcers > CRS-induced gastric ulcers. Both have shown similar degree of efficacy in almost all the models. Results clearly implied that the three combinations A1-50 mg/kg (25 mg of FRFE + 25 mg of AMRE), A2-75 mg/kg (37.5 mg of FRFE + 37.5 mg of AMRE) and A3-100 mg/kg (50 mg of FRFE + 50 mg of AMRE) show significant anti-ulcer effect in CRS-induced ulcer model. **Summary:** Combination of both FRFE and AMRE have exhibited remarkable anti-ulcer effect at even lower doses of equal proportions (1:1) in comparison with individual treatment studies, which also have shown good efficacy but much higher doses are required. This study highlights the importance of combined herbal formulations that help to facilitate the efficacy as well as safety of drugs.

Key words: *Aegle marmelos*, anti-ulcer effect, *Ficus racemosa*, gastroprotection

INTRODUCTION

The integrity of the gastric mucosa depends on the balance between aggressive (HCl, pepsine) and protective factors (mucus and HCO^3 - secretion, prostaglandins, mucosal blood flow, nitric oxide).[1] Therefore, whether the treatment is effective depends not only on the blockade of acid secretion, but also on the increased production of factors responsible for protecting the gastric mucosa, thus avoiding damage to the epithelium.[2]

Ficus racemosa Linn. (Moraceae) is an evergreen, moderate to large sized spreading, lactiferous, deciduous tree without much prominent aerial roots, found throughout greater part of India in moist localities and is often cultivated in villages for its edible fruit. All parts of this plant (leaves, fruits, bark, latex, and sap of the root) are medicinally important in the traditional system of medicine in India.[3] Previous studies reported the biological activities like hypoglycaemic,[4] anti-pyretic,[5] and anti-tussive.[6]

Aegle marmelos Corr. (Rutaceae) commonly called as "Bael" in Hindi is indigenous to India. It is a medium sized, armed, deciduous tree found wild, especially in dry forests and is also cultivated throughout Indian subcontinent for its fruit. The ripe fruit is used for digestive and stomachic complications. Leaves, fruits, stem and roots of *Aegle marmelos* have been used in ethno-medicine for several medicinal properties. Previous studies reported the various biological activities like hypolipidaemic,[7] immunomodulatory activity,[8] and anti-hyperglycaemic activity.[9]

In the traditional system of medicine, combined extracts of plants and herbal formulation are used as drug of choice rather than single drug. In this context, the present study has been designed to carry out comparative and combined anti-ulcer studies of *Ficus racemosa* Linn. (Gular) and *Aegle marmelos* (Bael) and to scientifically validate the traditional claims of the same.

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Address for correspondence: Dr. Siva Reddy Challa, Department of Pharmacology, KVSR Siddhartha College of Pharmaceutical Sciences, Pinnamaneni Polyclinic Road, Siddhartha Nagar, Vijayawada – 520 010, Andhra Pradesh, India. E-mail: sivareddypharma@gmail.com
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MATERIALS AND METHODS

Chemicals

Umbelliferone, gallic acid, thiobarbutric acid (TBA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), 5, 5 -dithiobis-2-nitrobenzoic acid (DTNB) and phenazonium methosulphate (PMS) were purchased from Sigma and Aldrich (St. Louis, MO, USA). Other chemicals and solvents were purchased from Merck Chemicals, Mumbai, India.

Animals

Sprague Dauley (SD) rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200–250 g were used. Animals were maintained under standard laboratory conditions at 25±2°C, 50±15% RH and normal photoperiod (12 h dark/ 12 h light). Commercial pellet diet (Rayon's Biotechnology Pvt. Ltd, Hyderabad India) and water were provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee and by the Animal Regulatory Body of the Government (Regd. No. 993/a/06/CPCSEA).

Plant Material

Ficus racemosa fruits, *Aegle marmelos* roots, leaves were collected from the botanical garden of our institute on 21/09/2009 and voucher specimens (NBR-89543 and NBR-89551) were preserved in departmental herbarium (Pharmacognosy and Ethnopharmacology Division) for future reference.

Preparation of Extract of *Ficus racemosa*

The unripe fruit (*Ficus racemosa* L.) (3 kg) was chopped into small pieces and dried under shade/tray drier under controlled conditions and powdered coarsely. Air-dried powdered fruits of *Ficus racemosa* Linn (1000 g) were powdered and exhaustively extracted by overnight maceration with 10 volumes of 50% ethanol and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on Rota vapour (Lukens Drive, USA) and then dried in lyophiliser (Freezone Plus 6, Labconco, USA) under reduced pressure to obtain 92.0 g of solid residue (yield 9.2 % w/w).

Preparation of Extract of *Aegle marmelos*

Roots (200 g) were washed with distilled water (dH_2O) to remove dirt and soil and were properly dried in shade for 4–7 days, then dried in tray drier maintained at 40°C. After drying, the plant materials were milled to powder and passed through the sieve (mesh size 40). The powdered materials were mixed in 50% ethanol solution for 2 days. The extract was separated by filtration and concentrated on Rota vapour (Buchi, USA) and then dried in lyophiliser (Labconco, USA) under reduced pressure to obtain solid residue (Root yield 3.2% w/w).

HPTLC Analysis

The ethanolic extract obtained was subjected to the preliminary photochemical studies for the presence of alkaloids, carbohydrate, flavonoids, glycosides, proteins and free amino acids, gums and mucilages, saponins, sterols, fixed oils, phenolic compounds and tannins using standard methods (Kokate, 1994). HPTLC analysis of F*icus racemosa* fruit extract (FRFE) was performed on pre-activated (100°C) silica gel $60F_{254}$ HPTLC plates (Merck) along with gallic acid and ellagic acid. Plates were eluted in solvent system toluene: ethyl acetate: formic acid (5:4:1) for phenols. After development, the plates were dried and densitometrically scanned at wavelength 366 nm (WinCats software, (Camag, Switzerland). HPTLC analysis of *Aegle marmelos* root extract (AMRE) was performed on pre-activated (100°C) silica gel $60F_{254}$ HPTLC plates (Merck) along with Umbelliferone. Plates were eluted in solvent system Toluene: ethyl acetate: formic acid (8:2:0.01) for phenols. After development, the plates were dried and densitometrically scanned at wavelength 366 nm (WinCats software, CAMAG, Switzerland).

Pylorus Ligation Induced Ulcers

Gastric ulcers were produced in rats by following method as described earlier by Sanyal (10). Briefly, the rats were fasted for 24 h before Pylorus Ligation (PL) but water was allowed *ad libitum*. At the end of 24 h starvation, rats were anaesthetized with pentobarbitone sodium (35 mg/kg). Abdomen was opened by a midline incision and a ligature was placed at the pyloric end of the stomach taking care not to exclude any blood vessels. The abdomen was then closed in two layers and rats were left in a cage with a false bottom of wide mesh wire gauze to prevent coprophagy. Water was withheld from 1 h before PL and until the end of 4 h period. Then, the rats were sacrificed by overdosing with ether. Immediately afterwards, abdomen was again opened, cardiac end of stomach was ligated and the stomach was taken out. The stomach was then cut open along the greater curvature and the mucosa was washed under slow running tap water. The ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows: 0, no ulcer; +, pinpoint ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1–2 mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and haemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated according to the method of Sanyal *et al*.[10]

Aspirin Induced Ulcers

Aspirin (ASP) was administered orally on the day of experiment with the help of an orogastric tube in the form of an aqueous water suspension (200 mg/kg, p.o.) and animals were sacrificed 4 h after drug administration. The stomach was incised along with the greater curvature and examined for ulcers as described earlier.[11]

Cold-restraint Stress Induced Ulcers

Rats of either sex weighing 120–150 g were immobilized for 2 h at 4°C following the method of immobilization as described earlier by Amar and Sanyal (1981) (12). Briefly, the animals were starved for 24 h with free access to water and 60 min after receiving the corresponding treatment, they were fully stretched and strapped to a wooden plank with adhesive tape after securing each limb to the plank individually. The animals were killed after 2 h and ulcer were scored as described above.[12]

Ethanol Induced Ulcer

The gastric ulcer was induced in rats by administering Ethanol (EtOH, 100%, 1 mL/200 g, 1 h). EtOH was administered on the day of the experiment and the animals were sacrificed by cervical dislocation and stomach was incised along with greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcer present in the glandular portion of the stomach (mm^2/rat).[13]

Gastric Secretion and Gastric Mucosal Studies

Collection of gastric juice and mucosal scrapings

The gastric juice was collected 4 h after PL and centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as mL/100 g body weight. The mucosal scrapings were taken from the glandular portion of the stomach and were homogenized in distilled water (10 mg/mL) to be used for various biochemical estimations.

Total acidity

Total acidity of the gastric juice was estimated by titration with 0.01 N NaOH using phenolphthalein as the indicator. The development of pink colour indicates the end point. The result was expressed either as mEq/mL or mEq/4 h for acid concentration and output, respectively.

Other gastric secretion parameters

Peptic activity was determined using haemoglobin as the substrate as standardised in this laboratory and reported earlier.[14] The dissolved mucosubstances in the gastric juice or gastric mucosal homogenate were estimated in the alcoholic precipitate obtained by adding 90 % alcohol in 9:1 ratio as described earlier.[10,11] Total hexoses were estimated using the method developed by Winzler.[15] Hexosamine was estimated using method developed by Dische and Borenfreund.[16] Fucose was estimated using method developed by Dische and Shettles.[17] Sialic acid was estimated using method developed by Warren (1959).[18] The protein content of the gastric juice was estimated using the method of Lowry.[19]

Malondialdehyde levels and Catalase in gastric tissue

The fundic part of the stomach was homogenized (5%) in ice-cold 0.9% NaCl with a Potter-Elvehjem glass homogeniser for 30 s. The homogenate was centrifuged at 800 x g for 10 min and the supernatant was again centrifuged at 12,000 × g for 15 min and the obtained mitochondrial fraction was used for the estimations of oxidants and anti-oxidant enzymes (19). Lipid peroxidation (LPO) in terms of malondialdehyde levels was estimated using method described by Ohkawa *et al*.[20] Catalase was estimated using the method described by Aebi *et al*.[21]

Acute Toxicity Studies of *Ficus racemosa* and *Aegle marmelos*

Various doses (50–2000 mg/kg, p.o.) of extracts of *Ficus racemosa* fruit and *Aegle marmelos* root and leaf were administered to group of rats and observed continuously for 1 h and at half-hourly intervals for 4 h, for any gross behavioural changes further up to 72 h, and followed 14 for days for any mortality.[22]

Experimental Design for *in vivo* Anti-ulcer Studies

The animals (SD rats 140–180 g) were divided into five groups of six animals each for individual treatment studies as well as combined effect study. Detailed experimental design is described in Table 1. Disease control group of animals received suspension of 1% carboxymethyl cellulose in distilled water (10 mL/kg). The standard drug used is sucralfate 250 mg/kg in these studies. A dose-response anti-ulcer study has been done using 100, 200 and 400 mg/kg of EtOH extract of *Ficus racemosa* Linn. and *Aegle marmelos* individually against various validated gastric ulcer models like PL, ASP, Cold-restraint Stress (CRS) and EtOH-induced ulcers. The 50% ethanolic extract

Table 1: Description of experimental design for *in-vivo* anti-ulcler studies in male SD rast

Groups	FRFE study	AMRE study	Combination (FRE+ AME) study
I	Disease Control	Disease Control	Disease Control
II	FRFE – 100 mg/kg, p.o.	AMRE – 100 mg/kg, p.o.	A1 Combination – (FRFE 25 mg + AMRE 25mg)
III	FRFE –200 mg/kg, p.o.	AMRE – 200 mg/kg, p.o.	A2 Combination – (FRFE 25 mg + AMRE 25mg)
IV	FRFE – 400 mg/kg, p.o.	AMRE –400 mg/kg, p.o.	A3 Combination – (FRFE 25 mg + AMRE 25mg)
V	Sucralfate – 250 mg/kg, p.o.	Sucralfate –250 mg/kg, p.o.	Sucralfate – 250 mg/kg, p.o.

FRFE – *Ficus racemsoa* fruit extract; AMRE – *Aegle marmelos* root extract

of respective plants were administered to various groups, orally twice daily for 5 days, and experiments were carried out on 18–24 h fasted rats on 6th day. Ulcers were scored and analysed as described earlier. In these studies, dose of 400 mg/kg has shown higher degree of efficacy and hence, 400 mg/kg dose was chosen for further subsequent studies on other parameters of gastric secretion or mucosal studies.

Statistical Analysis

Values from *in vitro* antioxidant and *in vivo* activities shown in Tables and Figures were the mean standard error mean (SEM) of at least three determinations and mean±SEM for six animals, respectively. Analysis was performed using one-way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison test (software "Prism 5.0") was applied for determining the statistical significance between different groups. The results were judged significant, if $P<0.05$, $P<0.01$ and $P<0.001$.

RESULTS

HPTLC Analysis

Results of phytochemical screening of the FREE and AMRE were presented in Table 2. The quantitative HPTLC determination shows the presence of anti-oxidants like gallic acid and umbelliferone in plant extracts of FRFE and AMRE, respectively. HPTLC fingerprint profiles of 50 % Ethanolic fruit extract of FRFE and AMRE at 366 nm are shown in Table 3. Both plant extracts were found to be safe up to 2000 mg/kg with no sign of mortality or change in behavioural pattern. These results suggest that the plant extracts are not toxic and to be safe.

Effect on Ulcer Index

Results of ulcer index are presented in Figure 1 and results of percent protection of gastric ulceration are presented in Figure 2. Effects of FRFE and AMRE at 100, 200 and 400 mg/kg, twice a day for 5 days significantly ($P<0.001$) prevented the acute gastric ulcers in a dose-related manner. Both ethanolic FRFE and AMRE have demonstrated the similar degree of anti-ulcer effect in different models of ulcer in the following order: PL-induced gastric ulcers > ASP-induced gastric ulcers > EtOH-induced gastric ulcers > CRS-induced gastric ulcers. The effect of combinations of these two plant extracts with a dose ratio of (1:1) was demonstrated on experimental models of ulcer and diabetes. Results were presented in Figure 3. The results clearly implied that the three combinations

Table 2: Preliminary phytochemical screening of the 50% ethanolic extract of FRFE and AMRE

Constituents	Tests	FRFE	AMRE
Carbohydrate	Molish's test	+	+
	Fehling's test	+	+
Proteins and amino acids	Million's test	+	+
	Ninhydrin test	+	–
	Biuret test	+	–
Phenolic compounds	$FeCl_3$ test	+	+
	Gelatin test	+	+
	Lead acetate test	+	+
Flavonoids	Aqueous NaOH test	+	+
	Con. H_2SO_4 test	+	+
Alkaloids	Mayer's test	+	+
	Dragendroff's test	+	+
Fixed oil and fats	Spot test	+	+

FRFE – *Ficus racemosa* extract; AMRE – *Aegle marmelos* extract; +Presence, –Absence

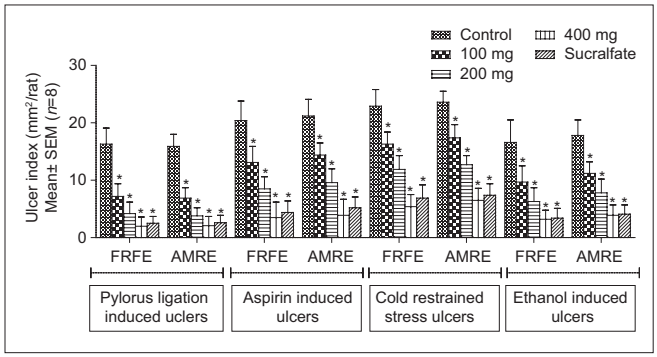


Figure 1: Effect of FRFE and AMRE on ulcer index in various experimental models of ulcer in male SD rats. All values are expressed as mean±SEM (n=8). FRFE-*Ficus racemosa* fruit extract, AMRE-*Aegle marmelos* root extract

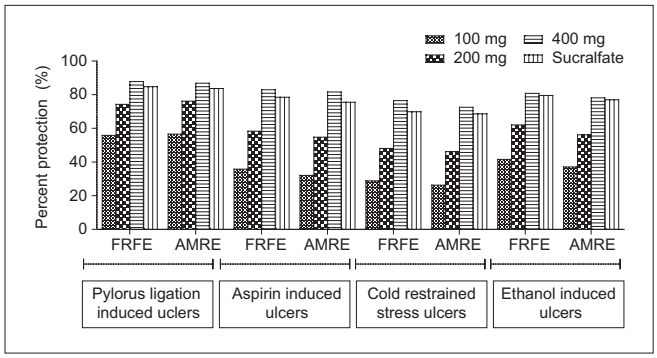


Figure 2: Percentage gastro-protection by FRFE and AMRE in various experimental models of ulcer in male SD rats. All values are expressed as mean±SEM (n=8). FRFE-*Ficus racemosa* fruit extract, AMRE-*Aegle marmelos* root extract

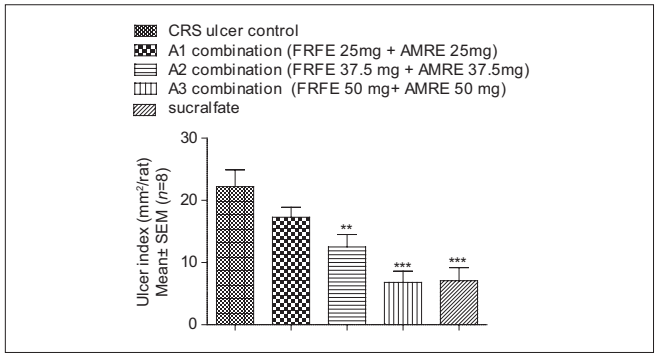


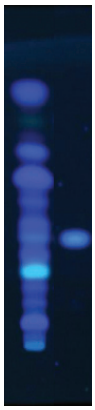
Figure 3: Effect of various combinations (1:1) of FRFE and AMRE on ulcer index in various experimental models of ulcer in male SD rats. All values are expressed as mean±SEM (n=8). FRFE – *Ficus racemosa* fruit extract, AMRE – *Aegle marmelos* root extract

A1-50 mg/kg (25 mg of FRFE + 25 mg of AMRE), A2-75 mg/kg (37.5 mg of FRFE + 37.5 mg of AMRE) and A3-100 mg/kg (50 mg of FRFE + 50 mg of AMRE) show significant anti-ulcer effect in CRS-induced ulcer model.

Effect on Volume, Acid and Pepsin Secretion

The effect of EtOH extract of FRFE and AMRE (400 mg/kg) when administered orally, twice daily for 5 days was studied for their effect on volume, acid and pepsin secretion in 4 h PL rats. FRFE and AMRE showed a tendency to decrease in volume the acid-pepsin concentration and output. However, reference drug, sucralfate, a known cytoprotective agent has little or no effect on volume, acid and pepsin concentration and acid output, but showed a significant decrease in pepsin output [Table 4].

Table 3: HPTLC finger print profile of 50% Ethanolic fruit extract of *Ficus racemosa* and root extract of *Aegle marmelos*

HPTLC finger print profile of 50% Ethanolic fruit extract of *Ficus racemosa* at 366 nm (Gallic acid as standard)		HPTLC finger print profile of 50% Ethanolic root extract of *Aegle marmelos* at 366 nm (umbelliferone as standard)	
Rf value		Rf value	
0.3		0.09	
0.5		0.16	
0.78		0.21	
0.82		0.25	
0.86		0.29	
		0.34	
		0.43	
		0.49	
		0.57	
		0.63	
		0.68	
		0.72	
		0.81	
	366 nm	0.87	UV-366 nm

Effect on Mucin Secretion

Mucoprotein was estimated in the 90 % alcoholic precipitate of the gastric juice in 4 h PL rats treated with FRFE and AMRE. The herbal drug showed a tendency to increase the concentration of total carbohydrates and individual carbohydrates like total hexoses, hexosaamine, fucose and sialic acid with a tendency to decrease protein content leading to a significant increase in TC:P ratio, indicating an increasing in mucin secretion, which was comparable with the effect of sucralfate on mucin secretion [Table 5].

Effect on Mucosal Glycoprotein

Gastric mucosal glycoproteins were studied in the 90% alcoholic precipitate of the homogenates of gastric mucosal scraping of the rats treated with FRFE and AMRE (400 mg/kg, twice daily for 5 days) or with a known cytoprotective drug, sucralfate (250 mg/kg, twice daily for 5 days). The result indicated a tendency to increase in the concentration of individual carbohydrates or total carbohydrates with a little change in protein level

Table 4: Effect of FRFE and AMRE on gastric secretion parameters like acid volume, acid concentration, acid output, pepsin concentration, pepsin output in cold restraint stress induced ulcer model in male SD rats

Parameter	Control	FRFE (400mg/kg)	AMRE (400mg/kg)	Sucralfate
Gastric secretion parameters				
Acid volume (ml/100g)	2.47±0.017	2.04±0.028***	2.24±0.030***	2.11±0.019***
Acid concentration (μEq/ml)	98.1±5.5	70.6±4.9***	74.8±3.6**	86.0±3.1
Acid output (μEq/4h)	284.4±18.9	144.0±10.7***	151.4±16.8***	181.5±19.3***
Pepsin conc. (μmol/ml)	295.3±11.5	286.1±13.6	274.6±12.8	204.8±19.6***
Pepsin output (μmol/4h)	729.4±29.3	383.6±19.3***	402.8±22.6***	453.2±19.7***

All values are expressed in mean±SEM (n= 8); **P<0.01, ***P<0.001 Vs control group by one way ANOVA followed by Tukey's test; FRFE – *Ficus racemsoa* fruit extract; AMRE – *Aegle marmelos* root extract

Table 5: Effect of FRFE and AMRE on mucin secretion parameters in cold restraint stress induced ulcer model in male SD rats

Parameter	Control	FRFE (400mg/kg)	AMRE (400mg/kg)	Sucralfate
Mucin secretion parameters				
Mucoprotein (μg/ml)				
Total hexose (A)	260.8±19.3	343.3±19.5**	351.4±11.5**	304.3±14.1
Hexosamine (B)	170.6±11.8	184.7±14.2	190.2±13.2	180.9±10.1
Fucose (C)	62.3±4.4	70.3±5.9	74.1±4.6	69.8±6.8
Sialic acid (D)	26.2±3.8	35.2±2.3	37.4±1.8*	36.0±2.3
TC (A+B+C+D)	519.9±23.6	633.5±29.3*	653.1±21.2**	519.0±26.3
Protein (P)	534.4±21.5	450.1±21.3	434.5±19.8	391.3±16.7
TC:P	1.05±0.011	1.48±0.015***	1.50±0.016***	1.53±0.016***

All values are expressed in mean±SEM (N= 8); *P<0.05, **P<0.01, ***P<0.001 Vs control group by one way ANOVA followed by Tukey's test; FRFE – *Ficus racemsoa* fruit extract; AMRE – *Aegle marmelos* root extract.

leading to an increase in total carbohydrate:protein ratio and, thus, mucosal glycoprotein in the treated groups [Table 6].

Effect on Malondialdehyde, Catalase in Gastric Tissue

The results of the present study on free radical-mediated lipid peroxidation and alteration in circulating enzymatic antioxidant enzymes like Catalase (CAT) indicate the involvement of these enzymes in ulcer. Malondialdehyde (MDA) was significantly increased in ulcer models and treatment with extracts have significantly (P<0.001) reduced the elevated levels of MDA. CAT was significantly depleted in ulcer control that was restored to normal with the treatment of plant extracts [Figures 4 and 5].

DISCUSSION

PL-induced ulcers are due to autodigestion of gastric mucosa and breakdown of gastric mucosal barrier.[23] ASP has been reported to produce ulcer both by local and systematic effects. Aspirin has direct irritant effect by increasing H^+ ions transport. EtOH causes ulcers by superficial damage to mucosal cells.[24] Stress plays an important role in etiopathology of gastrodueodnal ulcer. Increase in gastric motility, vagal overactivity, mast cell degeneration,[25] reduced gastric mucosal blood flow[26] and decreased prostaglandin synthesis due to stress induces ulcers.

The present study showed that the ethanolic FRFE and AMRE possess gastroprotective activity as evidenced by significant inhibition in formation of ulcers induced by various chemical and physical agents. Literature shows tannins may prevent ulcer development due to their vasoconstricting effects.[27] Recent work with unripe fruit of 50 % ethanolic extract of *Aegle marmelos* reported the presence of tannins and responsible for anti-ulcer activity.[6] Flavonoids reported to posses the property of preventing the formation of ulcers produced by various ulcerogens. In accordance with the previous study,[28] AMRE showed significant dose-dependent ulcer-protective effect against cold necrotic agents. Antioxidants prevent the ulcers formation caused by various ulcerogens.[29]

Both ethanolic FRFE and AMRE have demonstrated the efficacy in different models of ulcer in the following order: PL-induced gastric ulcers > ASP-induced gastric ulcers > EtOH -induced gastric ulcers > CRS-induced gastric ulcers. FRFE has shown much better efficacy than AMRE in these models of ulcer. Our results support the previous results reported that the fruit extract of *Aegle marmelos*

Table 6: Effect of FRFE and AMRE gastric mucosal glycoprotein parameters in cold restraint stress induced ulcer model in male SD rats

Parameter	Control	FRFE (400mg/kg)	AMRE (400mg/kg)	Sucralfate
Gastric mucosal glycoprotein				
Glycoprotein (μg/100mg wet tissue)				
Total hexose (A)	2524±183	3754±288**	3689±268*	3402±242
Hexosazmine (B)	1664±106	2239±230	2318±246	1986±143
Fucose (C)	319±22	381±30	396±24	379±27
Sialic acid (D)	119±13	176±16	184±14	243±33
TC (A+B+C+D)	4626±319	6550±399**	6587±319**	6010±343*
Protein (P)	6406±387	6328±453	6219±426	5793±293
TC:P	0.77±0.010	1.08±0.010***	1.05±0.012***	1.05±0.007***

All values are expressed in mean ± SEM (N= 8); *P<0.05; **P<0.01; ***P<0.001 Vs control group by one way ANOVA followed by Tukey's test; FRFE – *Ficus racemsoa* fruit extract; AMRE – *Aegle marmelos* root extract

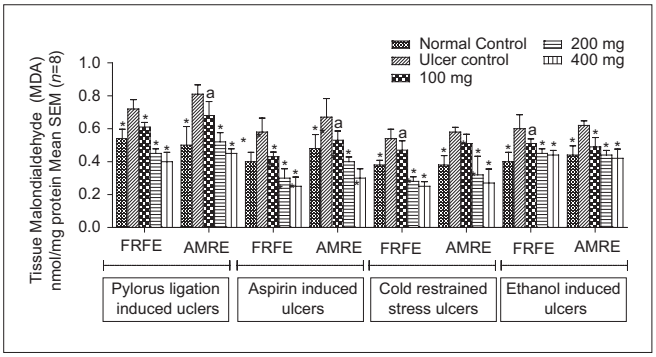


Figure 4: Effect of FRFE and AMRE on tissue malondialdehyde (MDA) levels in various experimental models of ulcer in male SD rats. All values are expressed as mean±SEM (n=8). FRFE-*Ficus racemosa* fruit extract, AMRE-*Aegle marmelos* root extract

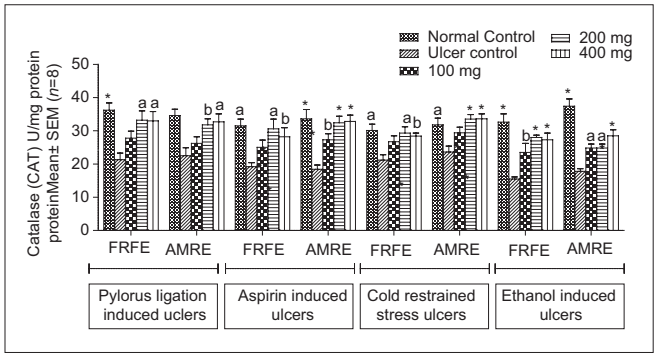


Figure 5: Effect of FRFE and AMRE on tissue catalase (CAT) in various experimental models of ulcer in male SD rats. All values are expressed as mean±SEM (n=8). FRFE-*Ficus racemosa* fruit extract, AMRE-*Aegle marmelos* root extract

exerted a significant protection against acute and chronic ulcers in rats.[6] Both FRFE and AMRE have exhibited the dose-dependent anti-ulcer activity with the doses of 100 mg/kg and 200 mg/kg whereas 200 mg/kg and 400 mg/kg have shown almost equal efficacy in the all the experimental models of ulcer. The dose of 400 mg/kg has produced maximal anti-ulcer efficacy. Hence, this dose of FRFE and AMRE was used for the assessment of parameters of gastric secretion or mucosal studies. Anti-ulcer activity was clearly evident from the reduced gastric secretion parameters and elevated mucoprotective parameters.

Bagchi *et al*,[30] hypothesised that free radicals may play a major role in stress-involved gastrointestinal injury. Stress is also found to inactivate mucosal prostaglandin synthetase by accumulating H_2O_2, an inhibitor of the prostaglandin synthesis, which propitiates the generation of reactive oxygen species. The process of lipid peroxidation is mediated by the interaction of hydroxyl radicals with the cell membrane, subsequently producing lipid-derived free radicals such as conjugated dienes and lipid hydroperoxides that cause oxidative damage.[31] In our results, the MDA levels, which indicate lipid peroxidation of the membranes, were significantly increased after CRS, which is closely related to tissue damage. *Ficus racemosa* and *Aegle marmelos* revert the content of TBA-reactive substances almost near to normal levels. Preventive antioxidants such as SOD and CAT enzymes are the first line of defence against reactive oxygen species. In our study, the administration of *Ficus racemosa* and *Aegle marmelos* combated CRS-induced gastric lesions and significantly increased CAT activity. This indicates that *Ficus racemosa* and *Aegle marmelos* protect the stomach by preserving antioxidant enzyme activity in the mucosa exposed to damage and hence the observed anti-ulcer activity of FRFE and AMRE are probably attributed to observed *in vivo* anti-oxidant activity of FRFE and AMRE in the gastric tissue.

The effects of combinations of these two plants' extracts with a dose ratio of (1:1) were demonstrated on experimental models of ulcer and diabetes. So the results clearly implied that the three combinations A1-50 mg/kg (25 mg of FRE + 25 mg of AME), A2-75 mg/kg (37.5 mg of FRE + 37.5 mg of AME) and A3-100 mg/kg (50 mg of FRE + 50 mg of AME) show significant anti-ulcer effect in CRS-induced ulcer model. Dose-dependent effect was clearly evident as the combined dose proportions were increasing.

In conclusion, these two extracts of FRFE and AMRE have demonstrated high synergistic gastro-protective activity even at low combinations of doses. This study highlights the importance of combined herbal formulations that help to facilitate the efficacy as well as safety of drugs in a given patient.

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