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Comparative evaluation of antiulcer activity of combination of extracts of *Embilica officinalis* Gaertn and *Musa sapientum* Linn in rats

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ABSTRACT

The present study is to evaluate the anti-ulcer activity of methanolic extract of unripe fruits of *Musa Sapientum* Linn. and the fresh juice of the fruits of *Emblica Officinalis* Gaertn in Wistar rats. The methanolic extract unripened fruits of *Musa Sapientum* Linn. and the fresh juice of the fruits of *Emblica Officinalis* Gaertn were combined and evaluated for the gastroprotective activity by ethanol-induced model and indomethacin-induced model in rats at a dose of 25mg/kg (p.o) and 50mg/kg (p.o) respectively. Biochemical parameters like ulcer index, the volume of gastric juice, pH of gastric juice, free acidity and total acidity were studied. The unripe fruits of *Musa* and the fresh juice of *Embilica* will contain flavonoids, flavones, abscisic acid which shows a significant reduction in gastric volume, pH, free acidity, total acidity and ulcer index at the doses of 25mg/kg (p.o) and 50mg/kg (p.o). The results show that the unripe fruits 0f *Musa Sapientum* Linn. and fresh juice from the fruits of *Emblica Officinalis* Gaertn will combinedly possess greater antiulcer activity than that of the individual drugs.

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INTRODUCTION

Among the gastrointestinal disorders, peptic ulcer is one of the disorders largely seen in adult males. Women seem to be peculiarly immune to peptic ulceration during the childbearing age (Parmar *et al.,* 1993). Gastric ulcers occur more frequently in the old age group. Duodenal ulcers occur commonly in younger individuals. The cause of the peptic ulcer was not known. Peptic ulcers are due to imbalances in mucosal aggressive and self-protective factors (Brodie, 1968). The main causes of gastric ulcers are acid secretion, cellular

regeneration, mucus secretion, blood flow, mucosal barrier, prostaglandins, and bacterial infections (Shay *et al.,* 1945). A dull and gnawing ache is the most common symptom which comes and goes for several days to weeks and occurs 2-3 hrs after a meal (Lee *et al.,* 1971). The symptoms include weight loss; poor appetite, bloating, nausea and vomiting. Emergency symptom includes the bloody or black stools that may accompany sudden and persistent stomach pain (Hoogerwerf*et al.,* 2006).

Various plants and herbs are used to treat gastrointestinal disorders in traditional medicine. Considering the side effects of ulcers in modern

medicine, indigenous drugs with fewer side effects are the better alternatives for the treatment of peptic ulcer (Dharmani *P et al.,* 2005). *Musa Sapientum* Linn. (Musaceace) is also known as Arati in India. It is indigenous to Bihar and Eastern Himalayas and is cultivated in tropical regions. It is rich in abscisic acid, alanine, arabinitol, benzaldehyde, flavones, leucine, isoleucine, lauric acid, palmitic acid, tryptophan, and valine (Goel R. K, 2002). It is used as an antiallergic agent, smooth muscle stimulant, uterine stimulant, anti-hypertensive (Alvarez, 2004). *Embilica officinalis*Gaetrn (Phylanthaceae) is known as Usiri in India. It is indigenous to Deccan, sea coast districts of Kashmir, tropical and sub-tropical parts. The fruit is rich in Vitamin C (Goel R. K, 1989). The seeds will contain a fixed oil, phosphatides and essential oils. Bark will contain the tannins. Roots contain ellagic acid and lupeol. The bark is rich in leucodelphin. It mainly contains the low molecular weight hydrolysable tannins namely embilican-A, embilican-B, pediculagin. It has been used in the indigenous system of medicine for aphrodisiac, antibacterial, anti-oxidant, diuretic, anti-diarrheal, anti-inflammatory, pruritus, brain tonic properties (Goel R. K, 1989).

MATERIALS AND METHODS

Collection of Plant Materials and Preparation of Extracts

The unripe fruits of *Musa sapientum* Linn. and fresh fruits of *Emblica Officinalis* Gaertn were collected and authenticated by Prof. Madhav Shetty, S. V. University, Tirupathi, Andhra Pradesh. The unripe fruits of *Musa sapientum*Linn were taken, peeled off, the pulp was taken, and it was cut into small pieces. The small pieces are air dried and powdered. The powdered pulp was exhaustively extracted with 95% methanol at room temperature for about 3 days. The methanolic solution was then filtered and concentrated under reduced pressure for dryness. The crude methanolic extracts were transferred into glass flasks and kept at 50^0C. The powdered methanolic exacts were dissolved in 25% methanol-water solution, then it was dried, and the final product is collected. Fresh fruits of *Emblica Officinalis* Gaertn were collected and crushed into small pieces. The seeds are removed from the fruits. The crushed pieces were passed through the juice extractor. The extracted juice was collected and passed through the filters for double filtration. The filtered juice was collected and stored in refrigerator at-20°C.

Animals

Wistar Albino rats weighing 150-200 gms of both the sex were used for the present study. The rats were maintained under standard environmental conditions and were fed with standard pellet diet and water ad libitum. The experiments were performed followed by approval from the animal ethical committee of the establishment IAEC 1521/PO/a/11/CPCSEA. The groupings of animals were done as mentioned below.

Group-1: Vehicle induced control
Group-2: Ethanol / Indomethacin control
Group-3: Animals were treated with *Musa sapientum* Linn. extract (p.o.) 25mg/kg
Group-4: Animals were treated with *Emblica officinalis* Gaertn. Extract (p.o) 50mg/kg
Group-5: Animals were treated with a combination of *Musa* and *Embilica* extracts (p.o.) 25mg/kg +50mg/kg respectively.
Group-6: Animals were treated with Ranitidine (p.o.) 80mg/kg respectively.

Methodology

Method-I Ethanol-induced method

The animals have fasted for about12 hours, and they were subjected to induce the ulcers with ethanol (1.5ml orally). After two hours of induction of ulcers with ethanol, the animals were sacrificed, and the stomachs of the rats were cut open along the greater curvature and parameters of gastric ulcers were checked (Turner, 1993 and Okabe *et al.,* 1970).

Method-II Indomethacin-induced model

The animals were to be administered with the vehicle and the extracts for about 14 days. After dosing for 14 days they fast for 36 hrs, then they were ulcer induced with indomethacin 30mg/kg orally to induce the ulcers. After 6 hrs of indomethacin induction the animals were sacrificed, the stomachs are cut open along the greater curvature and parameters of gastric ulcers were checked (Turner, 1993 and Okabe *et al.,* 1970).

Determination of anti-ulcer activity parameters

Ulcer index

The rats were sacrificed, and the dissected stomachs are cut open along the greater curvature, and the ulcer index was calculated from the glandular portions of the stomach (Turner, 1993).

The ulcer index was calculated as;

Ulcer index = 10/X
Where X= total mucosal area / total ulcerated surface.

The number of ulcers is noted, and the severity is recorded according to the scores given below:

0 = No visible ulcers
1 = ten or less small ulcers, 1-3 mm in diameter
2 = eleven or more ulcers, 1-3 mm in diameter
3 = one or more ulcers, 4-6 mm in diameter
4 = one or more ulcers, 7 mm or more in diameter
5 = perforation of the gastric wall

Volume and pH of gastric secretions

In a graduated test tube the contents of the dissected stomachs were taken and are allowed to centrifuge at 200rpm for 10min. The supernatant liquid was measured for the volume of gastric juice and expressed as ml/4hrs and pH of the gastric juice was measured.

Gastric acidity

In the conical flask, the supernatant liquid of the gastric juice was taken, and two drops of topfer's reagent were added. In a burette 0.01N, NaOH was added and allowed to triturate till the flask changed to yellow colour. Then two drops of phenolphthalein were added and triturate till orange colour was reached.

RESULTS AND DISCUSSIONS

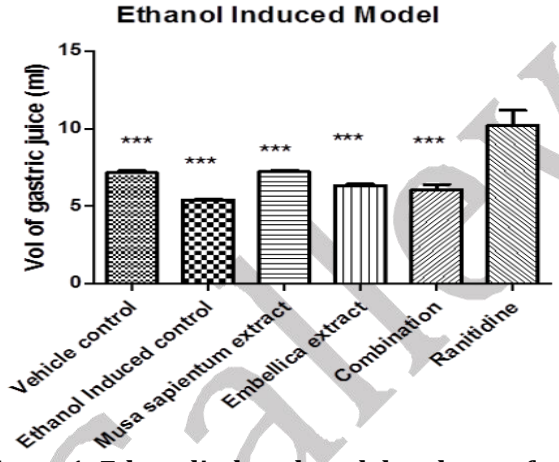

Figure 1: Ethanolinduced model- volume of gastric acidity significance values

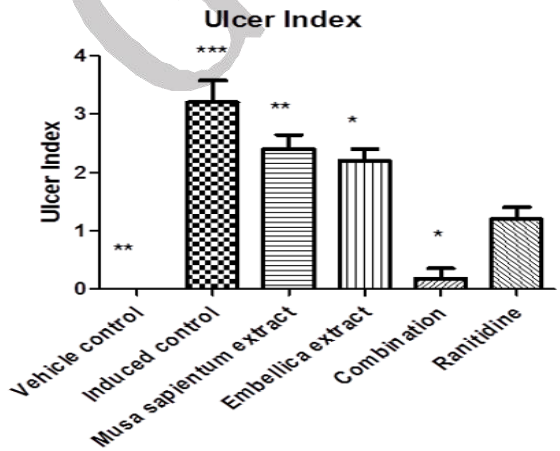

Figure 2: Ethanol-induced ulcers- Ulcer index significance values

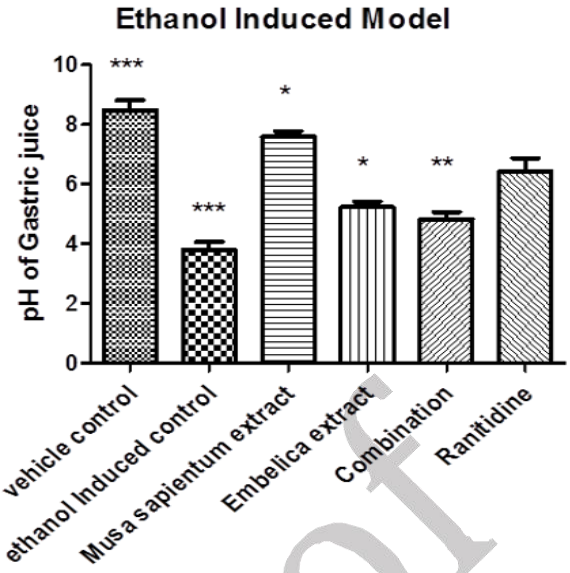

Figure 3: Ethanol-induced model – pH of gastric juice Significance values

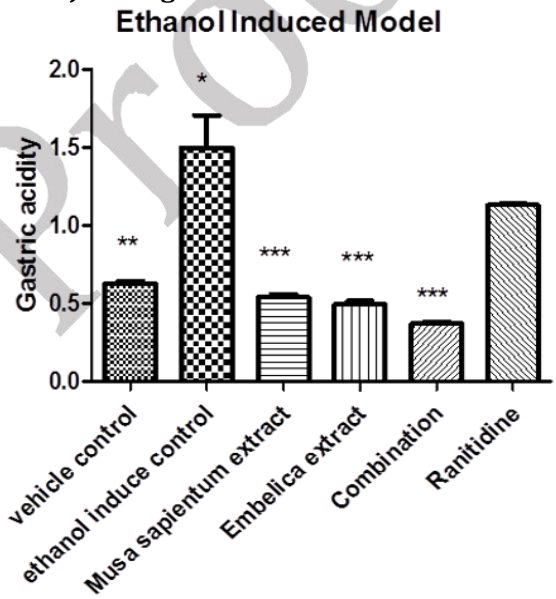

Figure 4: Ethanol-induced – gastric acidity significance values

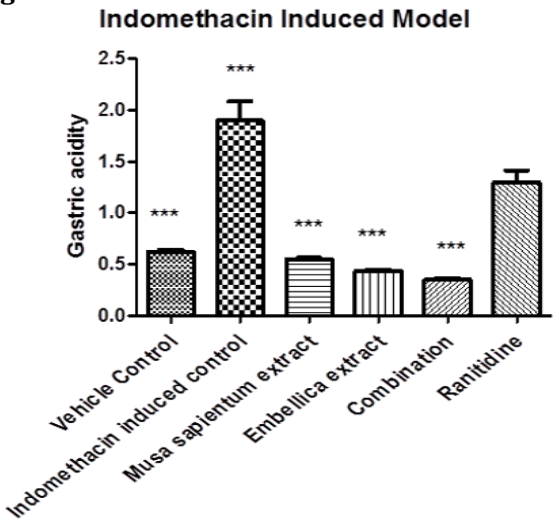

Figure 5: Indomethacin-induced model – Gastric acidity significance value

Table 1: Mean and SEM of ulcer index, the volume of gastric juice, pH of the gastric juice, gastric acidity for ethanol-induced ulcers

Treatment	Dose mg/kg	Ulcer index	Volume of gastric juice	pH of the gastric juice	Gastric acidity
Vehicle control	0.0mg/kg	0.000±0.000	7.16 ±0.116	8.480±0.333	0.630±0.0164
Ethanol-Induced control	1.5ml(p.o)	3.200±0.374	5.400±0.663	3.800±0.2702	1.500±0.2057
Extract-I (*Musa Sapientum* Linn.)	25mg/kg (p.o.)	2.400±0.244	7.220±0.1068	7.600±0.1817	0.5420±0.0174
Extract-II (*Emblica Officinalis* Gaetrn.)	50mg/kg (p.o.)	2.200±0.200	6.340±0.081	5.240±0.177	0.496±0.0218
Combination	25mg/kg (p.o)+ 50mg/kg (p.o.)	0.180±0.180	6.060±0.3516	4.840±0.2337	0.372±0.011
Ranitidine	80mg/kg (p.o.)	1.200±0.200	10.22±0.964	6.440±0.441	1.132±0.0106

Results are expressed as mean ± SEM; P* significance value <0.05 as compared to control

Table 2: Mean and SEM of ulcer index, the volume of gastric juice, pH of the gastric juice, gastric acidity for indomethacin-induced ulcers

Treatment	Dose mg/kg	Ulcer index	Volume of gastric juice	pH of the gastric juice	Gastric acidity
Vehicle control	0.0 mg/kg	0.000±0.00	6.48±0.066	9.240±0.261	0.620±0.023
Indomethacin Induced control	25 mg/kg (p.o)	3.800±0.374	4.600±0.167	4.380±0.153	1.900±0.187
Extract –I (*Musa Sapientum* Linn extract)	25mg/kg (p.o)	0.500±0.000	7.720±.0.243	8.260±0.128	0.552±0.011
Extract-II (*Emblica Officinalis* Gaertn extract)	50mg/kg (p.o)	0.600±0.100	7.680±0.086	6.660±0.136	0.436±0.012
Combination	25 +50mg/kg (p.o)	0.300±0.200	6.340±0.211	5.680±0.149	0.352±0.016
Ranitidine	80mg/kg (p.o)	1.400±0.187	8.460±0.250	7.560±0.186	1.300±0.110

Results are expressed as mean ± SEM; P* significance value <0.05 as compared to control

The ulcers induced by the ethanol are multifactorial. By using ethanol factors involved in the formation of ulcers shows that gastric wall depletion is one of the pathogenic mechanisms for gastric ulcers. For the determination of the ulcer index, the diameter of the lesions is considered. Non-steroidal anti-inflammatory drugs like indomethacin induce the punctiform and piliform ulcers. The indomethacin-induced ulcers are due to the inhibition of prostaglandin synthesis.

The observations in the present study as shown in the Figure 1, 2, 3, 4, 5, 6, 7 and 8 suggested that there is a significant decrease in the ulcer index, volume of the secretions, pH of the gastric juice of the extract due to the anti -secretary and the cytoprotective activity of the drug. As described in Table 1 and Table 2, the ulcer index, volume of the gastric juice, pH of the gastric juice, gastric acidity was decreased which indicates that increase in the mucous by protecting the gastric mucosa from ethanol-induced model and indomethacin-induced model of both the extracts are seen in this study.

CONCLUSION

Both the methanolic extracts of *Musa sapientum* and fresh juice of *Emblica Officinalis* have significant antiulcer activity, and their combination of methanolic extracts of *Musa sapientum* and fresh juice of *Emblica Officinalis*

shows the promising effects in decreasing the ulcers than that of the standard drug.

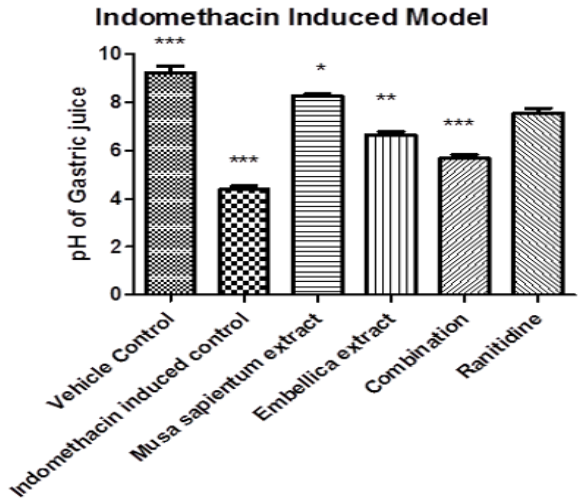

Figure 6: Indomethacin-induced model- pH of gastric juice significance value

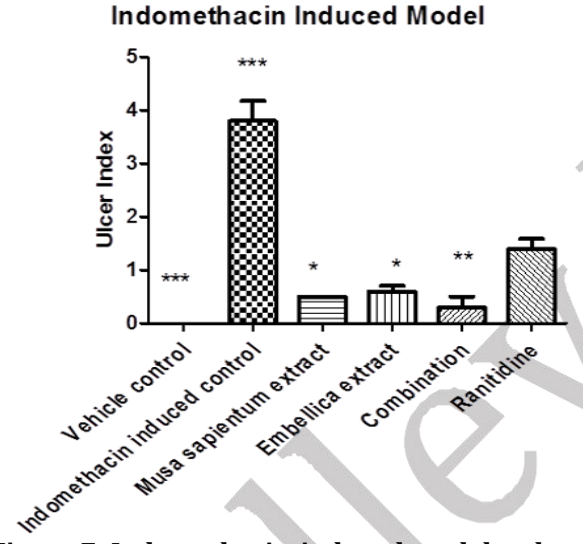

Figure 7: Indomethacin-induced model – ulcer index significance value

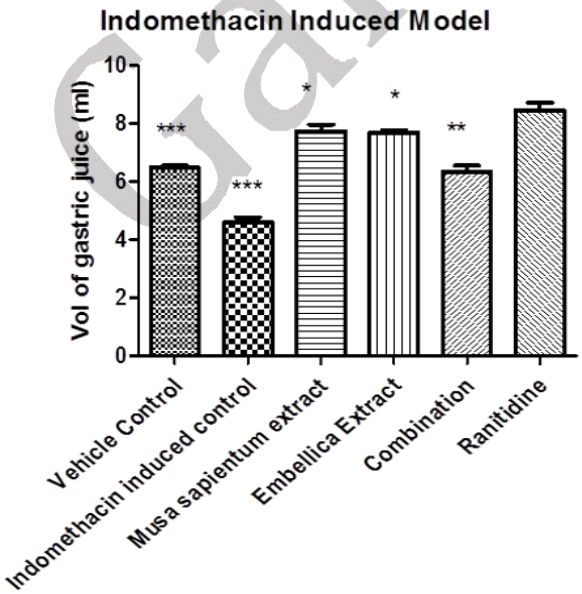

Figure 8: Indomethacin-induced model- Volume of gastric juice significance value

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