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Research Article

EVALUATION OF ULCEROPROTECTIVE ACTIVITY OF *MUSA SAPIENTUM* VAR. *PARADISIACA* METHANOLIC FRUIT EXTRACT AGAINST ASPIRIN INDUCED GASTRIC ULCERS IN ALBINO RATS

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ABSTRACT

Objective: The objective of the study was to evaluate the ulceroprotective activity of *Musa sapientum* var. *paradisiaca* methanolic fruit extract (MSE) against aspirin induced gastric ulcers in albino rats.

Methods: Fresh plantain bananas were sliced and air dried at room temperature. The dried slices were ground to a fine powder. The extract was prepared by percolating dried powder with methanol. Twenty four healthy albino rats of 100-200 gms were taken for the study. The animals were divided into four groups of six animals each. Group I or control (3% gum acacia 5 ml/kg orally for 7 days). Group II or experimental control (aspirin 400 mg/kg orally single dose on the 7th day). Group III or MSE group (MSE 100 mg/kg orally for 7 days and aspirin 400 mg/kg orally single dose on the 7th day). Group IV or standard (ranitidine 150 mg/kg orally for 7 days and aspirin 400 mg/kg orally single dose on the 7th day). On 8th day the stomachs of the sacrificed rats were removed and (1) ulcer index (2) total acidity (3) free acidity (4) gastric mucous secretion were studied.

Results: The ulcer index, free and total acidity in group III and IV showed significant decrease in comparison to group II (p <0.01) whereas there was increase in gastric mucus secretion (p <0.01).

Conclusion: The study showed significant ulceroprotective activity of *Musa sapientum* var. *paradisiaca* methanolic fruit extract (MSE) against aspirin induced gastric ulcers in albino rats.

Keywords: Aspirin, free acidity, gastric mucous, *Musa sapientum* var. *paradisiaca*, percolation, peptic ulcer, ranitidine, total acidity, ulcer index

Abbreviation: MSE – *Musa sapientum* var. *paradisiaca* extract.

INTRODUCTION

The term peptic ulcer refers to an ulcer in the lower oesophagus, stomach or duodenum, in the jejunum after surgical anastomosis to the stomach or, rarely, in the ileum adjacent to Meckel's diverticulum. Ulcers in the stomach and duodenum may be acute or chronic; both penetrate the muscularis mucosa but the acute ulcer shows no evidence of fibrosis [1]. It occurs due to imbalance between offensive (acid-pepsin secretion. *H. pylori*, bile, increased free radicals and decreased antioxidants) versus impaired mucosal resistance (mucous, bicarbonate secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury) [2]. Peptic ulcer therapy has undergone many strides over the past few years and a number of drugs are now available for treatment. These drugs are broadly classified into two, those that decrease or counter acid pepsin secretion and those that afford cytoprotection by virtue of their effects on mucosal defensive factors [2].

The traditional systems of medicines – *Ayurveda*, *Siddha* and *Unani* are based on the experiences in the use of plant products in amelioration of common diseases. A vast majority of our population particularly those living in villages depend largely on herbal medicines [3]. The importance of traditional system of medicine and of certain traditional medical practices has now been recognized all over the world [4].

Musa sapientum var. *paradisiaca* Linn. (plantain banana) belongs to family *Musaceae*. The plants are giant herbs with false aerial stems and sheathed leaves arising from a rhizome. Fruit is berry [5]. It is available worldwide. Different parts of the banana plant are used for various traditional medicinal purposes. Roots and stems are used as tonic, antiscorbutic, useful in blood and venereal diseases. Unripe fruit in combination with other drugs used in diabetes [6].

The plants contain tannic and gallic acids. The unripe fruit contains a flavonoid leucocyanidin [7]. The fruit is very rich in micro and macro nutrients. Unripe fruit contains high amounts of calcium and selenium. Ripe fruit contains aspartic acid, glutamic acid, Leucine etc [8].

The study aimed to evaluate the ulceroprotective activity of *Musa sapientum* var. *paradisiaca* methanolic fruit extract against aspirin induced gastric ulcers in experimental animals.

MATERIAL AND METHODS

The study was carried out in the department of pharmacology at Assam Medical College in 2006. Fresh plantain bananas were collected from areas near Assam Medical College campus, Dibrugarh, in the months from March to May 2006. A taxonomist of Dibrugarh University identified and confirmed the fruit samples.

The bananas were sliced and air dried at room temperature. The dried slices were ground to a fine powder. Methanolic extract was obtained by percolating the dried powder with 50% methanol [9].

Animals: The experiment was carried out in albino rats of the species *Rattus norvegicus* of either sex weighing 100-200 gms. All the animals were taken care of under ethical consideration with approval from the institutional ethical committee (Registration no.-634/02/a/CPCSEA), Assam Medical College, Dibrugarh. During the entire period of experiment, the animals were given standard diet with water *ad libitum*.

Acute toxicity studies: *Musa sapientum* var. *paradisiaca* methanolic fruit extract was subjected to acute oral toxicity as per OECD Guidelines 425 [10]. Mortality in the acute oral toxicity test was not seen in the limit test up to dose 2000mg/kg.

Materials required for the study: Drugs used in the study were *Musa sapientum* var. *paradisiaca* methanolic fruit extract, ranitidine, aspirin and vehicle - 3% gum acacia suspension for all preparations.

Experimental design: A total twenty four healthy albino rats of 100-200 gms were divided into four groups of six animals each. Group I or control was administered with 3% gum acacia 5 ml/kg

orally for 7 days. Group II or experimental control was administered with aspirin 400 mg/kg orally as a single dose on the 7th day of experiment. Group III or MSE group was administered with MSE 100 mg/kg orally for 7 days and aspirin 400 mg/kg orally as a single dose on the 7th day. Group IV or standard was administered with ranitidine 150 mg/kg orally for 7 days and aspirin 400 mg/kg orally as a single dose on the 7th day.

After administration of aspirin on 7th day to the groups II, III and IV, all the animals in the study were fasted overnight and on the 8th day pyloric ligation was done under light anaesthesia and kept for 4 hours [11]. After sacrifice of the animals, the stomachs were removed and opened along the greater curvature. The contents were collected in test tubes for estimation of (1) ulcer index (2) free acidity (3) total acidity (4) gastric mucus secretion.

Ulcer index: The resected stomachs of the sacrificed rats were opened along the greater curvature. The opened stomachs were given a gentle wash with a running stream of water. The stomachs were then placed on the card boards, luminal surface facing up. The ulcer index was then calculated from the glandular portion of the stomach, with the aid of a magnifying glass and measuring tape [12].

The ulcer index was calculated as:

$$\text{Ulcer index} = \frac{10}{x} \qquad \text{Where } x = \frac{\text{Total Mucosal Surface}}{\text{Total Ulcerated Area}}$$

Measurement rules: Each lesion was measured long the greatest length, in case of petechial, five of these were considered to be equivalent to 1 sq-mm of ulcer area, the total area of the glandular portion of stomach and that of ulcerated mucosa were measured for determination of the ulcer index [12].

Free acidity and total acidity: As described by Kulkarni SK (1999), after centrifuging the gastric contents, 1 ml of the supernatant was taken in a conical flask and diluted with distilled water to make a volume of 10 ml. 2 drops of Topfer's reagent was added to it. 0.01N NaOH was taken in a burette and allowed to titrate into the conical flask until the solution in the conical flask changed colour to yellow, or the methyl yellow end point. Then 2 drops of phenolphthalein was added and titration was continued till orange colour or phenolphthalein end point was reached [13].

The amount of 0.01N NaOH required to titrate to the methyl yellow end point is the measure of the free acid present. The amount of 0.01N NaOH required to titrate from the beginning to the phenolphthalein end point is a measure of the total acid present in the sample. The acidity was calculated by the following formula and expressed in mEq/l [13].

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality x 100}}{0.1} \text{ mEq/l}$$

Gastric mucus secretion: The quantitative estimation of gastric mucus was carried out according to Corne SJ et al (1974). Briefly excised glandular portion of stomach was soaked in 0.1% alcian blue solution buffered with 0.05M sodium acetate and HCl. Uncomplexed dye adhering to tissue was washed with 0.025M sucrose, tissue again soaked in $MgCl_2$. The blue solution thus obtained mixed with ether and optical density was measured against 605nm [14].

Statistical analysis: Statistical analysis in the present study was done by ANOVA followed by Dunnet's Multiple Comparison Test and Bonferroni Test. $p < 0.05$ was considered to be significant.

RESULTS

The present study showed that the *Musa sapientum* var. *paradisiaca* methanolic fruit extract significantly lowered ulcer index, pepsin activity, free acidity and total acidity but significantly increased gastric mucus secretion. Standard drug ranitidine also decreased ulcer index, free and total acidity, increased gastric mucous significantly.

From the observations in the present study it is evident that *Musa sapientum* var. *paradisiaca* methanolic fruit extract has significant ulceroprotective activity against aspirin induced gastric ulcers in experimental animals.

Table1: The effect of *Musa Sapientum* var. *paradisiaca* methanolic fruit extract on the ulcer index against aspirin induced ulcers in experimental animals (Values are expressed in Mean ± SEM)

GROUP	DOSE P.O.	ULCER INDEX
I (normal)	5 ml/kg	0.22 ± 0.01
II (aspirin)	400 mg/kg	19.24 ± 0.95[a]
III (MSE)	100 mg/kg	5.3 ± 0.46[b]
IV (ranitidine)	150 mg/kg	3.86 ± 0.23[b]
One Way ANOVA	F	235.52
	df	20, 3
	p	< 0.01

n = 6 in each group; [a] :→p < 0.01 when compared to normal control;
[b] : p < 0.01 when compared to experimental control; ANOVA followed by Dunnet's Multiple Comparison Test and Bonferroni Test.

Table2: The effect of *Musa Sapientum* var. *paradisiaca* methanolic fruit extract on free acidity against aspirin induced ulcers in experimental animals (Values are expressed in Mean ± SEM)

GROUP	DOSE P.O.	FREE ACIDITY (mEq/l)
I (normal)	5 ml/kg	53.25 ± 2.59
II (aspirin)	400 mg/kg	98.82 ± 2.30[a]
III (MSE)	100 mg/kg	71.31 ± 4.15[b]
IV (ranitidine)	150 mg/kg	65.40 ± 2.90[b]
One Way ANOVA	F	39.71
	df	20, 3
	p	< 0.01

n = 6 in each group; [a] :→p < 0.01 when compared to normal control;
[b] : p < 0.01 when compared to experimental control; ANOVA followed by Dunnet's Multiple Comparison Test and Bonferroni Test.

Table3: The effect of *Musa Sapientum* var. *paradisiaca* methanolic fruit extract on total acidity against aspirin induced ulcers in experimental animals (Values are expressed in Mean ± SEM)

GROUP	DOSE P.O.	TOTAL ACIDITY (mEq/l)
I (normal)	5 ml/kg	126.40 ± 3.80
II (aspirin)	400 mg/kg	220.20 ± 5.80[a]
III (MSE)	100 mg/kg	154.20 ± 2.50[b]
IV (ranitidine)	150 mg/kg	144.60 ± 1.50[b]
One Way ANOVA	F	116.94
	df	20, 3
	p	< 0.01

n = 6 in each group; [a] :→p < 0.01 when compared to normal control;
[b] : p < 0.01 when compared to experimental control; ANOVA followed by Dunnet's Multiple Comparison Test and Bonferroni Test.

Table4: The effect of *Musa Sapientum* var. *paradisiaca* methanolic fruit extract on gastric mucus against aspirin induced ulcers in experimental animals (Values are expressed in Mean ± SEM)

GROUP	DOSE P.O.	GASTRIC MUCUS (mg alcian blue/gm of glandular tissue)
I (normal)	5 ml/kg	1.80 ± 0.11
II (aspirin)	400 mg/kg	0.48 ± 0.03[a]
III (MSE)	100 mg/kg	1.00 ± 0.19[b]*
IV (ranitidine)	150 mg/kg	1.08 ± 0.01[b]
One Way	F	22.12

ANOVA	df	20, 3
	p	< 0.01

n = 6 in each group; [a] :→p < 0.01 when compared to normal control;
[b] : *p* < 0.01 when compared to experimental control; * : *p* < 0.01 when compared to ranitidine group; ANOVA followed by Dunnet's Multiple Comparison Test and Bonferroni Test.

DISCUSSION

Peptic Ulcer Disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder [15]. Recently there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most studies focus on newer and better drug therapy [16]. Till date only few documented studies of ulceroprotective activity of herbal drugs are available, like black turmeric or *Curcuma caesia* [17], *Spathodea falcate* [18] etc.

From the results of this study it is observed that *Musa sapientum* var. *paradisiaca* methanolic fruit extract (MSE) produces significant ulceroprotective effect against aspirin induced gastric ulcers in albino rats. The aetiology of gastric ulcers is not known in most cases, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms [19].

Aspirin induced ulcers cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ ions and thus leading to breaking up of mucosal barrier [20]. Aspirin increased ulcer index, free acidity, total acidity and reduced gastric mucous in the present study.

Pre-treatment with MSE (100 mg/kg) orally for 7 days showed significant reduction (p <0.01) of the aggressive factors i.e. free acidity, total acidity. MSE significantly increased (p <0.01) defensive factor i.e. gastric mucus. Increase in mucosal protective factors and decrease of aggressive factors may be responsible for the ulcer protective activity of *Musa sapientum* var. *paradisiaca.*

Banana is rich in various flavonoids. The major components of this group of polyphenolic compounds are the flavan-3'4-diols also known as leucoanthocyanidins [21]. Flavonoids are known to exhibit anti-inflammatory, anti-neoplastic, and hepatoprotective activities [22]. More recently they have been shown to reduce acid secretion from gastric parietal cells [23]. This shows that in the present study MSE exerted its ulceroprotective effect probably due to its flavonoid content.

Certain studies on herbal plants showed antioxidant mediated ulceroprotective activity in experimental animals [24, 25]. Antioxidant mediated antiulcer activity of the methanolic extract of banana was reported in a study [26]. Thus the antioxidant activity maybe one of the mechanisms through which banana exerts its ulceroprotective effect.

In the present study ranitidine (150 mg/kg) showed significant decrease in ulcer index, free acidity, total acidity and significant increase in gastric mucus. The effects of ranitidine and MSE were comparable in relation to free acidity, total acidity and ulcer index, whereas in case of gastric mucus ranitidine was found to be more effective than MSE.

CONCLUSION

The present study suggests that *Musa sapientum* var. *paradisiaca* methanolic fruit extract possesses significant ulceroprotective effect against aspirin induced gastric ulcers in experimental animals.

The observations of the present study puts forward *Musa sapientum* var. *paradisiaca* fruit as a promising new ulcero-protective agent, but further studies for detailed phytochemical composition, and studies with more refined techniques on animal & human subjects are required to establish the true potential in terms of therapeutic and economic viability of this herbal plant.

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