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THE EFFECT OF *MUSA SAPIENTUM* METHANOLIC FRUIT EXTRACT ON VOLUME OF GASTRIC JUICE AND PEPSIN ACTIVITY IN ASPIRIN INDUCED PEPTIC ULCERS IN ALBINO RATS

N. Barua*, M. Das and S. Das

Department of Pharmacology, Jorhat Medical College and Hospital, Jorhat- 785001, Assam, India

Keywords:

Pepsin activity, gastric juice, *Musa sapientum*, percolation, peptic ulcer, ranitidine, aspirin

Abbreviation:

MSE – *Musa sapientum* extract

Correspondence to Author:

N. Barua

Department of Pharmacology, Jorhat Medical College and Hospital, Jorhat- 785001, Assam, India

E-mail: drnilotpal@gmail.com

ABSTRACT: The aim of the study was to evaluate the effect of *Musa sapientum* methanolic fruit extract on volume of gastric juice and pepsin activity in aspirin induced peptic ulcer in albino rats. The extract was prepared by percolation method. Volume of gastric juice was estimated by the method of Deshpande SS *et al* (2003). Pepsin activity was studied with the methods of Debnath PK *et al* (1974) and Lowry OH *et al* (1951). Twenty four healthy albino rats of 100-200 gms were divided into 4 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. The volume of gastric juice and pepsin activity were studied. The volume of gastric juice and pepsin activity in group III and IV showed significant decrease in comparison to group II (p <0.01). Both *Musa sapientum* fruit extract and the ranitidine significantly (p <0.01) reduced volume of gastric juice and pepsin activity.

INTRODUCTION: A peptic ulcer disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder [1]. The aetiology of peptic ulcer is not clearly known. It results probably due to an imbalance between the aggressive and defensive factors. Reactive oxygen metabolites, free radicals, nitric oxide and genetic and environmental factors are also thought to play a role in the pathogenesis of ulcer [2].

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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 [3].

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 [4]. 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 are used in diabetes [5].

The plants contain tannic and gallic acids. The unripe fruit contains a flavonoid leucocyanidin [6]. 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. [7].

The study aimed to evaluate the effect of *Musa sapientum* methanolic fruit extract on the volume of gastric juice and pepsin activity in aspirin induced peptic ulcer in albino rats.

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 [8].

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*.

The methanolic extract of *Musa sapientum* fruit was subjected to acute oral toxicity as per OECD Guidelines 425 [9]. Mortality in the acute oral toxicity test was not seen in the limit test up to dose 2000 mg/kg.

A total twenty four healthy albino rats of 100-200 gms were divided into 4 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 [10]. After sacrifice of the animals, the stomachs were removed and opened along the greater curvature. The contents were collected in test tubes for analysis.

The methods of estimating volume of gastric juice was as per Deshpande SS *et al* (2003), [11] and pepsin activity was as per Debnath PK *et al* (1974) [12].

Volume of Gastric Juice: The contents of the resected stomachs of the rats were taken in graduated test tubes the test tubes were allowed to centrifuge at 2000 rpm for 10 minutes. The supernatant fluid was measured for volume of gastric juice and expressed as ml/4 hours [11].

Pepsin activity: The gastric contents were collected and centrifuged and 1 ml of the supernatant was used to study the pepsin activity [12].

1 ml of the gastric juice was diluted with 0.01M HCl (1: 250). Then 1 ml of the diluted gastric juice was mixed with 2.5 ml of 2% haemoglobin solution in 0.06M HCl. The mixture was allowed to incubate at 37^0C for 20 minutes and immediately thereafter, 0.6M ice cold trichloroacetic acid was added in equal volume. The tube containing this mixture was allowed to stand in an ice bath for 15 minutes. The mixture was then centrifuged for 10 minutes at 2000 rpm to separate the precipitated proteins.

0.6 ml of the clear supernatant thus obtained was used to determine the liberated amino acids [13].

In a 6 ml test tube, 0.6 ml of supernatant was taken. 3 ml of reagent C was added to the supernatant and allowed to stand for 10 minutes at room temperature. Then 0.3 ml of reagent E was added with constant stirring. The mixture was allowed to stand for 30 minutes. Thereafter the sample was read for optical density in a colorimeter at 610nm, against a blank similarly prepared with 0.01M HCl

instead of diluted gastric juice. The pepsin activity was expressed in terms of μmol tyrosine/ml of gastric juice.

Optical density (O.D.) due to the liberated amino acid by pepsin present in the gastric juice of the test sample – (Test – Control) = *X* (say).

Pepsin activity of diluted gastric juice:

$$= \frac{\text{Concentration Of Standard Tyrosine}}{\text{O.D. of Standard Tyrosine}} \times X = Y \text{ (say)}$$

The pepsin activity of undiluted gastric juice = *Y* x 251 x 7 μmol tyrosine/ml.

Statistical analysis: Statistical analysis was done using one way ANOVA followed by Dunnet's Multiple Comparison test and Bonferroni's test. Significance level of <0.01 was considered as significant.[14]

In this study, observations of table 1 and table 2 respectively showed that the methanolic extract of *Musa sapientum* fruit lowered the volume of gastric juice and pepsin activity significantly (p <0.01) compared to the control.

TABLE 1: EFFECT OF MSE ON THE VOLUME OF GASTRIC JUICE (Values are expressed in Mean ± SEM)

Group	Dose P.O.	Volume of Gastric Juice (ml/4 hours)
I (normal)	5 ml/kg	5.10 ± 0.32
II (aspirin)	400 mg/kg	8.30 ± 0.40[a]
III (MSE)	100 mg/kg	3.10 ± 0.12[b]
IV (ranitidine)	150 mg/kg	2.50 ± 0.10[b]
One Way ANOVA	F	110.86
	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.

TABLE 2: EFFECT OF MSE ON PEPSIN ACTIVITY (Values are expressed in Mean ± SEM)

Group	Dose P.O.	Pepsin Activity (μmol tyrosine/ml)
I (Normal)	5 ml/kg	101.60 ± 3.93
II (Aspirin)	400 mg/kg	353.42 ± 36.84[a]
III (MSE)	100 mg/kg	207.33 ± 6.66[b]
IV (Ranitidine)	150 mg/kg	186.22 ± 18.41[b]
One Way ANOVA	F	26.39
	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.

In the present study the volume of gastric juice was estimated by the method of Deshpande SS *et al* (2003).[11] The pepsin activity of this study was measured by methods of Debnath PK *et al* (1974)[12] and Lowry OH *et al* (1951) [13].

The volume of gastric juice was 5.10 ± 0.32 in the control group. The volume of gastric juice in the control group in the study of Deshpande SS *et al* (2003) was 8.85 ± 1.07. The volume of gastric juice was significantly increased (p<0.01) in the aspirin group when compared to the control group [11].

MSE and ranitidine significantly reduced (p<0.01) the volume of gastric juice, when compared to the aspirin group.

The pepsin activity (µmol tyrosine/ml) of the control group in the present study was 101.60 ± 3.93 which was comparable to the finding of Datta M *et al* (2002), who got pepsin activity of 60.3 ± 2.09 in 3 hours pyloric ligated control groups [15].

Aspirin treated group showed significant increase (p<0.01) in pepsin activity compared to the control.

The animals of the present study pre-treated with MSE (100 mg/kg) and ranitidine (150 mg/kg) showed significant reduction (p<0.01) in pepsin activity when compared to aspirin administered group.

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 [16]. Flavonoids are known to exhibit anti-inflammatory, anti-neoplastic, and hepatoprotective activities [17]. They have been shown to reduce acid secretion from gastric parietal cells [18].

The pepsin activity lowering effect of methanolic extract of *Musa sapientum* fruit was probably due to the presence of flavonoids [16]. The methanolic extract was also shown to have antioxidant mediated anti-ulcer activity [19].

Discussing the results in this study, it has been observed that the *Musa sapientum* methanolic fruit extract significantly lowered the volume of gastric juice and pepsin activity.

CONCLUSION: In conclusion, the observations of the present study puts forward *Musa sapientum* 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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