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EVALUATION OF GASTRIC ANTIULCEROGENIC ACTION OF VEGETABLE PLANTAIN BANANA (*MUSA SAPIENTUM* VAR. PARADISIACA) IN ASPIRIN PLUS PYLORUS LIGATED ALBINO RATS

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ABSTRACT

Objectives: To assess the effect of unripe dried banana powder in experimentally induced gastric ulcers and effect on gastric acid secretion. To evaluate the antiulcerogenic effect of plantain banana of Gujarat as a part of evaluation of impact of biological variables on this activity.

Materials and methods: Total of 24 albino rats of either sex weighing between 150-250 gm were randomly divided into 4 groups. Each group has 6 no. of rats. The first group received placebo (distilled water), the second, third & forth group received 0.5gm/kg, 1gm/kg and 2mg/kg of banana powder respectively. Banana powder was given as suspension at fixed time (3 times in a day) for two days and animals were kept for fasting for another 48hrs. On 5th day, the animals were sacrificed after 7 hrs and stomach were removed for examination and gastric juice samples were collected to analyze volume and acidity.

Results: Orally administered banana powder in the dose of 2gm/kg caused a statistically significant decrease in aspirin with pyloric ligation induced ulcers in rats without significantly decrease in secretary activity.

Conclusion: It can be concluded from these results that vegetable plantain banana has antiulcerogenic and mucosal protective actions, but it has no antisecretory activity.

INTRODUCTION: Peptic ulcer is a psychosomatic disease, a major, health problem in terms of morbidity and mortality. It is a worldwide problem and its prevalence is quite high in India. According to several field studies from different parts of our country, its occurrence is 4 to 10 per thousand populations. Three states of India viz. Tamil Nadu, Andhra Pradesh, and Jammu and Kashmir are considered to be very high risk areas [1].

It is a benign lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin. It occurs due to imbalance between offensive (acid-pepsin secretion. *H. pylori,* bile, increased free radicals and decreased antioxidants) versus impaired mucosal resistance (mucus, bicarbonate secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury) [2].

Initially for several decades the adage "no acid- no ulcer" and the drugs used to reduce acid secretion have dominated the pharmacological basis of ulcer therapy [3]. But then after the role of mucosal factor in peptic ulceration has received much attention and the term "cytoprotection" has been raised. It is now well established that peptic ulcer disease can be prevented by strengthening the defensive mechanisms of gastric and duodenal mucosa rather than attenuating factors of aggression causing ulceration [4].

Most commonly used drugs namely antacids, H_2-blockers and proton pump inhibitors etc. act by reducing the aggressive factors. Various reports have shown that these commonly used drugs for peptic ulcers have danger of drug interaction, adverse effects and increased incidence of relapses during ulcer therapy. Because of the problem of being highly complex, expensive and toxic effect of these agents, efforts were made to find a suitable palliative and /or curative agent for the treatment of peptic ulcer disease from natural products of plants and animal origin which afford better protection and decrease the incidence of relapse. Further, herbal drugs mostly augment the defensive factors such as mucin secretion, cellular mucus, bicarbonate secretion, mucosal blood flow and cell turnover [5].

Medicinal herbs are an indispensable part of the traditional medicine, practiced all over the world due to easy access, low cost and ancestral experience. The indigenous system in medicine makes a substantial contribution to the public health in India and other developing countries. Millions of household in rural and urban areas in our country consume traditional diet, use home remedies and health customs based on principles of traditional medicine. In traditional Indian medicine several plants and herbs have been used to treat gastrointestinal disorder, including peptic ulcer.

Various herbal drugs notably *Eugenia jambolana* (Jamun), [6] *Bacopa monnieri* (Linn.,) [7] *Ginkgo biloba,* [8] *Dhatura fastuos,* [9] *Coccinia grandis* (Linn.), [10] *Solanum nigrum, Brassica oleracea* and *Ocimum basilicum,* [11] Ginger (*Zingiber officinale* Roscoe) [12] etc. have been tried for their ulcer protective effects both experimentally and clinically (*Musa sapientum*) [13] and their effects seem to be due to their predominant effect on mucosal defensive factors.

Therefore, the search for an ideal antiulcer drug continues and has also been extended to herbal drugs for their better protection, easy availability, low cost and less toxicity.

Banana is a very common, large evergreen tree of Indian subcontinent. The term banana is Spanish – Portuguese from Guinea. Plantain refers in India to a coarse banana. Traditionally different parts of this plant are used namely root, leaf, fruit, stem, flowers for numerous purposes. That is why so many studies have been done to evaluate the different properties of banana. In addition to its nutritional value, a number of biological activity studies have been carried out on banana and these studies showed that this food plant has possessed bioactivities including anti-hyperglycaemic, anti-ulcerogenic, antioxidant, anti-hypertensive, cardiac depressant, diuretic, anti-tumoral, bronchodilatory, expectorant, oral contraceptive, abortifacient, antibacterial, antifungal etc.[14].

Earlier studies showed antiulcerogenic effect of unripe green banana against Phenylbutazone induced [15] and immobilization induced, [16] aspirin induced gastric ulcers in rats and histamine induced gastric ulcers in mice [17] and also work has been done to study the effect of different biological variables on the antiulcerogenic property of banana itself.

Biological variables like maturity, species, season, soil, etc., are well known to influence the biological activity of an herbal drug. A study has been done to evaluate the role of these variables. In those studies the vegetable plantain bananas were obtained from different states of the country viz., UP, Karnataka, Kerala, Maharashtra, W. Bengal and Tamil Nadu in the month between February and March [18]. So in the present study, we wanted to evaluate the antiulcerogenic effect of vegetable plantain banana of Gujarat.

MATERIALS AND METHODS: The experiments were carried out according to CPCSEA guidelines (Committee for the Purpose of Control and Supervision on Experiment on Animals) in the department of pharmacology, Shri M. P. Shah Medical College, Jamnagar.

1. **Source of Plantain Banana:** The bananas used in this experiment were unripe plantain bananas purchased from a local Jamnagar market in the month of February and it was used as suspension throughout the experiment.

2. **Preparation of Powder**: Skin from each fruit was peeled off and pulp was cut into thin slices. These slices were spread over plastic sheets and dried in sunlight. The dried slices were then milled to get the powder for experimental purpose.

3. **Animals**: Albino rats of *Charles Foster Inbred* strain of either sex weighing between 150-250g were used. They were fed standard rodent pellet diet and six rats were used in each group.

4. **Drugs/Reagents**: Topfer's reagent [(solution) Qualigens fine chemicals, Glaxo India Limited, Mumbai], Sodium hydroxide [(Pellets) M.W.40, Ranbaxy fine chemicals, S.A.S. Nagar] 0.01N solution was used, Phenolphthelein (I.P)- 1% Solution prepared in methanol. (1gm phenolphthalein dissolved in 100ml of methanol), Aspirin (uncoated tablets) - Obtained from Govt. hospital's (GGH, Jamnagar) pharmacy. Tablets were crushed and suspension was made with 1% Gum Acacia. 100mg of Gum Acacia was added in 10 ml of distilled water, Anaesthetic ether (TKM Pharma, Hyderabad). Thiopental sodium for injection (I.P) - Reconstitution was freshly made and 100mg/ml concentration was used.

Antiulcer studies:

Aspirin plus Pyloric Ligation (PL) model: Animals were housed under standard environmental conditions. Initially pilot study was done to standardize the dose of aspirin to produce significant ulcer. At the dose of 800mg/kg aspirin significant ulcers were produced. Then the rats were randomly divided into 4 groups. Randomization was done by draw system and the whole process was kept blinded. Group 1 served as control. Placebo (Distilled water 0.8ml/100gm of body weight of animal) was given at fixed time (3 times in a day) for two days. In the group II, III and IV banana powder was administered orally by gavage as suspension in the dose 0.5, 1 and 2 gm/kg body weight at fixed time (3 times in a day) for two days respectively.

The rats were kept for fasting for another 48hrs, care was taken to avoid coprophagy and water was provided ad libitum. On the 5th day, after 48hrs, pylorus was ligated as per the method described by shay under ether anaesthesia, then after the abdominal wall was closed by suturing and animal was allowed to recover from the anaesthesia. Aspirin suspended in 1% gum acacia was given orally to each animal 15 min after pyloric ligation. The animal was sacrificed after 7 hrs; the stomach was removed and washed with normal saline. Then small nick was put on the greater curvature slightly above the knot of pylorus and gastric contents were collected. Stomach was opened along greater curvature and wash gently with water and examined for severity of ulceration [19, 20].

Scoring (Ulcer Index): Severity of ulceration was graded according to the following scale which is a modification of score by Kunchandy *et al* [21]:

0 - Normal gray colored stomach

0.5 - Pink to red coloration of stomach

1 - Spot ulcer

1.5 - Hemorrhagic streak

2 - Number of ulcers less than 5

3 - Number of ulcer more than or equal to 5

4 - Ulcer with bleeding

5 - Perforation of gastric/duodenal wall.

Ulcer Index was calculated by adding the total number of ulcers plus the severity of ulcer.

Gastric Secretion Analysis: Gastric juice sample was collected and centrifuged for 10 minutes (3000 rpm) and the supernatants were collected and the following estimations were done in the gastric juice, obtained from the sacrificed animals. The volume of gastric juice was measured. A drop of the gastric juice was taken with a glass rod and placed on a strip of pH paper. The color change was match with the standard chart and reading was noted. The first indicator used in the titration is Topfer's reagent. Topfer's reagent changes the color yellow to red (orange) from pH 2.9 to 4.4. The second indicator Phenolphthalein changes the color yellow to red (pinkish) from pH 8.3 to 10.

The first titration to about pH 4 measures the amount of free hydrochloric acid present in the gastric juice. The complete titration gives the value for total acid. Acid output was determined by titration with N/100 NaOH using phenolphthalein as an indicator to light pink end point and expressed for concentration as millimoles per hour [22].

The person measuring the ulcers as well as gastric secretion was not aware of which animals had been treated.

Statistical Analysis: All data were expressed as mean± S.E.M. Groups of data were compared with one way analysis of variance (ANOVA) followed by student'*t*' test. Values of p< 0.05 were considered as significance.

RESULTS: Comparison among 4 groups was done with ANOVA test. F-value at 5% level of significance is 3.10. Since the computed F-ratio is greater than the critical ratio, the Mean Ulcer Index of 4 groups of rats differs significantly (**Table 1**).

TABLE 1: EFFECT ON ULCER INCIDENCE

Group	Severity of Ulcer (S.U.)	No of Ulcer (N.U.)	Ulcer index (S.U. + N.U.)	Mean ±SEM
Control	18	42	60	10
Banana powder (0.5 gm/kg)	16	35	51	8.50±1.52
Banana powder (1 gm/kg)	15.5	31	46.5	7.75±1.48
Banana powder (2 gm/kg)	8.5	11	19.5	3.25±1.59*
One way ANOVA	F- 3.132 p- 0.05			

* indicates level of significant as p < 0.05 compared to control group

The antiulcerogenic effect of orally administered banana powder prepared from plantain banana was studied against aspirin with pylorus ligated induced gastric ulcers in groups of rats and the results compared with control. In all animals of control group, aspirin with pyloric ligation for 7 hours produced deep and large ulcers in glandular portion of the stomach. Mean Ulcer Index amounted to 10, there was no perforation.

The animals, each consumed 0.5gm/kg (Group 2), 1gm/kg (Group 3) and 2gm/kg (Group 4) of plantain banana powder respectively before fasting and aspirin treatment. In group II and III, although it showed trend of decrease in ulcer incidence, it was not statistically significant. It was observed that in group IV, there was marked decrease in Ulcer incidence as compared to control and treated groups (0.5mg/kg and 1gm/kg). So severity of ulcer is much less with this dose, which was statistically significant (**Table 1 & Fig. 1**).

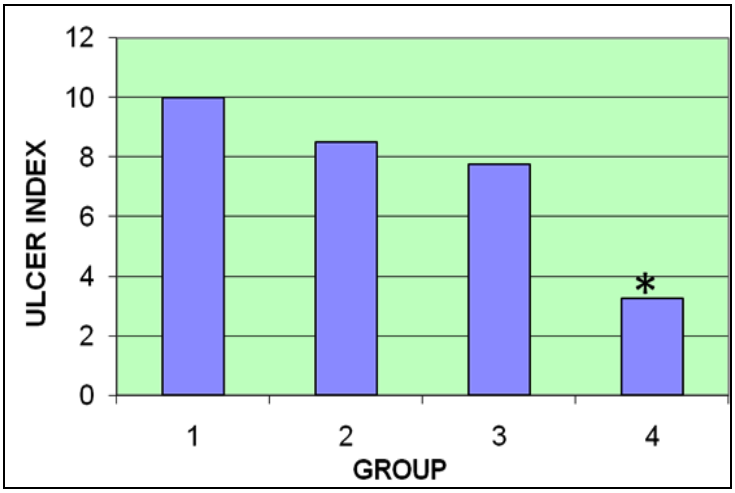

FIG. 1: COMPARISON OF ULCER INDEX BETWEEN CONTROL (GROUP 1) AND TEST (GROUP 2, 3, 4)

TABLE 2: EFFECTS ON GASTRIC ACID SECRETION

Group	Volume of gastric juice (ml)	Free acidity (mEq/L)	Total acidity (mEq/L)	pH
Control	5.38 ±1.66	19.7 ±6.5	81.3 ±8.5	4
Banana powder (0.5gm/kg)	6.00 ±0.62	19 ±0.93	63.5 ± 6	4
Banana powder (1 gm/kg)	4.6 ±0.99	15.3 ±6.10	62.7± 7.6	4
Banana powder (2 gm/kg)	5.58 ± 2.08	17.4 ±0.58	78.8 ± 12.8	4

Although results showed trend of decrease in acidity with all doses (0.5gm/kg, 1gm/kg and 2gm/kg) of banana powder but the difference was not statistically significant (**Table 2 & Fig. 2**).

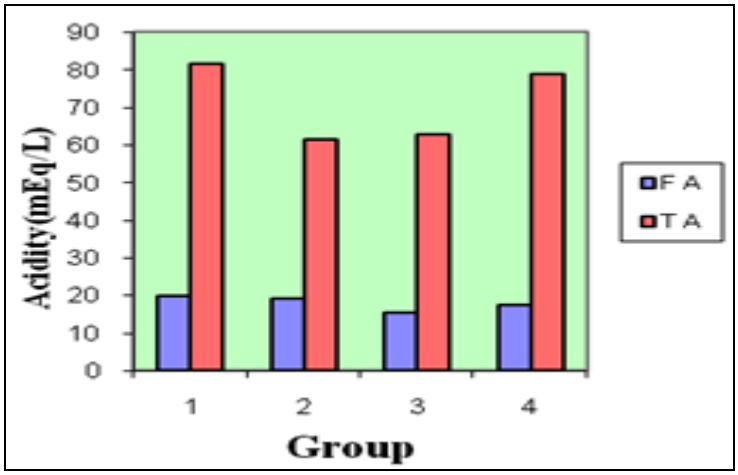

FIG. 2: COMPARISON OF TOTAL AND FREE ACIDITY BETWEEN 4 GROUPS

DISCUSSION: Medical intervention for non-steroidal anti-inflammatory drugs (NSAIDs) related mucosal injury, especially in patients with chronic therapy with NSAIDs such as in rheumatoid arthritis, includes treatment of an active ulcer and prevention of future injury. Cessation of NSAIDs is not always possible because of the severity of underlying disease. Nowadays mainly two drugs are used for this purpose; one is prostaglandin analogue (e.g. Misoprostol) which can only used as prophylactic therapy, after stopping of NSAIDs. But it has side effect like diarrhoea in majority of the cases and also some major toxicities like uterine bleeding and contraction. It is contraindicated in pregnant and women of childbearing age. [23]

One another group of drugs, which are extensively used, is proton pump inhibitors like omeprazole, pantoprazole and rabeprazole. These drugs can be used for prophylactic as well as curative therapy. But this drug is also not free from side effects. 5-10% cases of hypergastrinemia have been reported. Gastrin is a trophic factor for epithelial cells, and this may lead to different kind of tumors in the gastrointestinal tract.

In rats, undergo long term administration of proton pump inhibitor, there has been development of entero chromaffin like cell hyperplasia and gastric carcinoid tumors secondary to sustained hypergastrinemia. This has raised concerns about the possibility of similar complications in human. The development of a hypergastrinemic state may predispose the patient to rebound hypersecretion of gastric acid following discontinuation of therapy [3]. So, various problems are there with these commonly used drugs for peptic ulcers.

So it is justified to search for more and different agents those have antiulcerogenic activity which are relatively cheaper and safer than above mentioned drugs and also can be used for long term therapy.

We have used the aspirin with pyloric ligation model. Non-steroidal anti-inflammatory drugs can injure the gastro-duodenal mucosa by their topical and systemic effects causing direct tissue damage and through inhibition of prostaglandin (PG) synthesis, increasing acid secretion and back diffusion of H^+ ions. Similarly PL leads to stasis of secretion leading to acid induced mucosal damage [24].

In the present study, it was found that pretreatment with 0.5gm/kg and 1gm/kg banana powder produced lower no. of ulcers in the treated groups as compared to control group. However, the difference couldn't reach to statistical significance level. Probably because the sample size was small or the doses of 0.5 gm/kg and 1gm/kg are not adequate to produce complete therapeutic effect (Table 1). In the rats treated with 2gm/kg banana powder, marked protective effect against ulceration was demonstrated. The mean Ulcer Index of this group was significantly different ($p<0.05$) from that of control group (Table 1). This suggests this agent possibly has cytoprotective action and probably also increases mucosal resistant to damaging effects of drugs. There was no significant difference between group II and group III and also between group III and group IV. But difference between group II and group IV was statistically significance ($p<0.05$). So it suggests that there is significant difference in ulcer protection with doses 0.5gm/kg and 2gm/kg. So we assume that there might be ulcer protection in dose dependent manner.

It was found that in aspirin with pylorus ligated rats there was trend of decrease in total and free acidity with all three doses of banana powder as compared to control. But these differences couldn't reach to statistical significance level. There was no difference in volume of gastric juice among all these groups. So there is no effect of vegetable plantain banana on gastric acid secretion, which supports the conclusion of previous studies [18] that the antiulcerogenic effect was not due to its antisecretory and demulcent action.

Here, we have not explored the mechanism of antiulcerogenic activity of plantain banana. But various studies were carried out to explore the same. In one study ethanolic extract of banana was reported to increase the accumulation of eicosonoids like prostaglandins E and I2 (PGE and PGI2) and leukotrienes B4, and C4/ D4 (LTB4, C4/D4) in the human gastric and colonic mucosal incubates. PGs are reported to be one of the factors involved in the anti-ulcerogenic activity [25].

One experimental study proved that the antiulcer effect of banana was not due to its 5-HT content, but could be due to its predominant effect on mucosal defensive factors. It was reported that DRBP (Dried banana powder) have no activity on offensive acid pepsin secretion and the effect was mostly ascribed to increase in gastric mucus secretion quantified in terms of total carbohydrate:protein ratio (TC:PR ratio)[18] Further studies with DRBP on the changes induced by ulcerogenic agents like aspirin, indomethacin, phenybutazone and prednisolone in the dissolved mucosubtances of gastric juice showed that it not only increased the TC:PR ratio of the gastric juice, but also reversed the decrease in ratio induced by ulcer producing drugs [26].

It was also observed that significant decrease in protein content of the gastric juice while, there was no change in the individual carbohydrates leading to increase in TC: PR ratio. This decrease in protein content signifies decreased leakage from gastric mucosa indicating increased strengthening of gastric mucosal barrier. Increase in glycoprotein content of the mucosa and cell shedding in the gastric juice were also reported [27]. Apart from mucosal resistance DRBP was also reported to increase cell proliferation as observed from increase in mucosal thickness and in DNA and [3H]-thymidine uptake by the mucosal cells. This property was also reported to be contributed in healing of ulcers [28].

The clinical usefulness of DRBP was also investigated by using radiological and endoscopic studies. It was found to decrease or delay the relapse of peptic ulcer to 6-12 months after 3 months continuous treatment with DRBP, when given in the dose of 1 g *q.i.d*.

Furthermore, double blind study done at many centers have shown that about 40-70% of endoscopically proved duodenal ulcers healed after 12 weeks of treatment with DRBP, as compared to about 16 % with placebo [29]. In another clinical study with DRBP in non-ulcer dyspepsia (NUD), it have been reported that relief in symptoms by 75% after 8 weeks of treatment compared to only 20% in the controls [30].

The results of the above studies with dried plantain banana pulp powder, therefore makes it a potent herbal drug for the treatment of peptic ulcer disease and prompts that chemistry of banana pulp should be studied extensively to find out the active principle(s), which can be promising ulcer healing drug/s. Till then one should not hesitate to use its dried powder in the treatment of peptic ulcer disease as the powder seemed to be potent and safe.

Limitations: Limitations of our study were:-

- Sample size was small.
- Limited parameters were used.
- Only one animal model was used.

However, since the study was blind one and all precautions taken to avoid human error or bias makes us feel that results are dependable and it needs further study for its ulcer protective action.

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