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Cytoprotective Effects of Musa Paradisiaca and in Combination with Catecholamines on Indomethacin-Induced Peptic Ulceration in Rats

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ABSTRACT

This study is designed to investigate the cytoprotective effect of the methanolic extract of *Musa paradisiaca* in combination with catecholamines on indomethacin-induced peptic ulcer. The pylorus ligation technique was used for cytoprotective and Anti-secretory action of the extract. Ulcerated control received distilled water, Group II - IV received 0.5 ml of the plant extract orally for 14 days. The rats were fasted for 48 hours after the end of the second week. 50mg/kg of adrenalin (Epinephrine) was administered to members of group II and 50mg/kg of dopamine was administered to members of group III. One hour later the animals were sacrificed, the stomachs were removed by laparastomy. The gastric lesions in the glandular region were assessed and measured to determine the ulcer index. Pylorus ligation in the group II, III and IV showed significant ($p < 0.05$) reduction in the ulcer index compared to group I. The ulcer index of Group I was 14.8±3.5 compared to Group II (8.2±1.4), Group III (4.8±1.7), and Group IV (3.0±1.1). The extract also showed 67.57% ulcer protection index against indomethacin. Results of the anti-secretory activity of the extract showed that treatment with *M. paradisiaca* resulted in a significant increase in gastric fluid after histamine stimulation when compared with the negative control as well as protect rats from ulceration after histamine administration. The results suggested that the methanolic extract of *Musa paradisiaca* possess cytoprotective effect against indomethacin-induced ulceration. This effect was however decreased when the extract is administered with catecholamines.

Keyword: Ulcer, catecholamine, *Musa paradisiaca*, indomethacin, gastroprotection, pyloric ligation technique

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INTRODUCTION

Gastro duodenal ulceration is a common disease in both developed and developing countries[1, 4]. Many factors have been implicated in the development of gastric ulcers. Among these factors is smoking, caffeine, alcohol, stress, Helicobacter pylori and non steroidal anti inflammatory drugs (NSAIDs) such as aspirin, Ibuprofen and naproxen sodium[2]. Most of these factors either weakens the stomach's protective mucous and make it more susceptible to the damaging effect of acid and pepsin or stimulate the secretion of gastric acid[3].The hypersecretion of gastric acid has been discovered to be a strong factor in the development of both acute and chronic gastric mucosal lesions[4]. This is shown in the fact that suppression of gastric acid by surgical and pharmacological means provides effective and rapid healing of ulcers[5].

Recent studies with plantain (*Musa paradisiaca*) have indicated its ulcer-protective and healing activities through its predominant effect on various mucosal defensive factors[6, 7, 8]. The extract showed significant antiulcer effect and antioxidant activity in gastric mucosal homogenates, where it reversed the increase in ulcer index, lipid peroxidation and super oxide dismutase values induced by stress[9, 10, 11].Also because the use of herbs and herbal therapies in Africa and other part of the world including the U.S is escalating, it is essential to be aware of clinical and adverse effects, doses and potential drug interactions[12, 13]. Most practicing physicians have little knowledge of herbal treatments or adverse effects. A consumer poll in 1998, however, indicated that nearly one-third of respondents use botanical remedies[14] .Moreover, of those who take prescription medications, nearly one in five uses herbs, high-dose dietary supplements or both[15], suggesting an estimated 15 million adults are at risk for adverse interactions involving prescription medications and herbs or vitamin supplements.

In Africa, there has been many sponsored ethnobotanical survey which revealed the presence of many plants purported by traditional practitioners to be efficient in the management of complications arising from peptic ulcer[16].This study is under taken to determine if the medicinal properties of unripe plantain extract alone and in combination with catecholamine can prevent ulcer induced by indomethacin in rats.

MATERIALS AND METHOD

Location of study

This study was carried out at the Department of Human Physiology, Madonna University, Elele campus, Rivers State, Nigeria. The University is located on an elevation of about 120m above sea level at latitude $5^0$21'North and Longitude $7^0$29'East. Elele falls within the rainforest zone of

Nigeria which is characterized by hot and humid climate. The mean annual rainfall is about 2177mm, mean annual relative humidity is about 90% and that of temperature is 22^0C to 32^0C depending on the season.

Plant material

Fresh fruits of *Musa paradisiacal* were obtained from local vendors in Rivers State and identified at the Department of Botany and Microbiology, Madonna University, Nigeria. The fruits of *Musa paradisiacal* were peeled and the pulps were sliced and sun dried for ten days. The dried pulps were pounded into powder form. The pounded material weighing 500g was stored in air-tight bottles for extraction. 150g of the powdered material was packed into a soxhlet apparatus and extracted by maceration with 350ml of methanol for 48 hours. The methanol was evaporated using a rotary evaporator (Model 349/2 Corning, England). The filtrate was further concentrated using an electric incubator. The extract came as semi-solid greenish brown paste. A stock solution was afterward prepared by dissolving 100g of the extract in 50 ml of distilled water.

Phytochemical screening

The extract was evaluated for the presence of flavonoids, glycosides, carbohydrate, protein, tannins, alkaloid, saponins and sterols/triterpenes using method described by Brain and Turner[17].

Acute toxicity test (Median Lethal dose, LD_{50})

This was performed on mice and the Lorke[18] procedure of LD_{50} determination was used. The mice were randomly allocated into 5 groups of 3 animals each. Groups 1, 2, 3 and 4 were treated with 100, 500, 1000 and 1500 mg/kg of extracts of *M. paradisiaca,* respectively. Group 5 received normal saline and served as untreated control.

Animal monitoring and feeding

A total of 32 mature wistar rats, weighing about 150 – 250g, were used for this study. The hutches for the animals were thoroughly cleaned and disinfected. The rats were raised in the animal house of the Faculty of Basic Medical Sciences of Madonna University, Rivers, Nigeria and fed with rodent diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. The animals were kept at a temperature of 25˚C to 30˚C and 60 – 65% humidity with 12 hours light and dark cycle. The experimental protocols and procedures used in this study were approved by the Ethical Committee, Madonna University, Nigeria and conform to the guideline of the care and use of animals in research and teaching[19].

Animal grouping

The rats were deprived of food 24 hours before the experiment but allowed water ad libitum and were divided into four (5) groups containing five rats, as follows;

Group 1: This served as the control cage. Members of this group were fed with normal rat chow and water *ad libitum* for two weeks of the experiment.

Group 2: This group of rats was fed with the normal rat chow and 50 mg/kg of the extract for the two weeks of the research. Feeding stops 48 hours prior to the end of the second week. 50 mg/kg body weight of a 100% solution of epinephrine was injected intraperitonially into the rats 30 minutes before ulcer induction. Ulcer induction was by administration of indomethacin (Strides, Belgium) dissolved in 1% sodium hydrogencarbonate and administered orally (40mg/kg) to the fasting rats 30 minutes after administration of test drug (epinephrine; Tonogen R, Ritcher Gideon, Budapest Hungary) and were then placed under anesthesia 4 hours after gastric secretion and ulcer was monitored and studied thereafter.

Group 3: The rats in this group were fed as in group 2 except that a Dopamine (Tonogen R, Ritcher Gideon, and Budapest Hungary) concentration of 50 mg/kg body weight of a 100% solution was injected into the rats in place of epinephrine.

Group 4: The same as in groups 2 and 3 except that there was no administration of catecholamine into the rats before ulcer induction.

Sample collection and assessment

After the fourteenth day, the animals were euthanized and their stomach opened along the greater curvature. The stomach of each animal was rinsed under a stream of saline and pinned flat on a cork board. The ulcers were viewed with the aid of a magnifying lens (10X) and were given a severity rating[20]as follows:

<1mm=1 (pin point)

>1mm<2mm=2

>2mm<3mm=3

The overall total divided by a factor of 10 was designated as the ulcer index (UI) for that stomach. The surface area of each lesion was also determined as well as the percentage ulcerated surface.

The percentage inhibition was calculated using:100 – (A/B x 100/1)

Where,

A = Group treatment mean value (ulcer index)

B =Control mean value (ulcer index)

Determination of anti-secretory activity

To determine anti-secretory activity, twelve rats were grouped into three groups (I – III) of four rats each. The rats were fasted for 36 hours prior to the test. The 36-hr fasted rats were anaesthetized under thiopental anesthesia, the abdomen cut open, the stomach brought out and the pylorus ligated. Care was taken to avoid bleeding or occlusion of the blood vessels. The incisions were carefully sutured after doses of *M. paradisiacal*(200mg/kg), saline (5ml/kg), and Cimetidine (100mg/kg) were administered intra-peritoneally, immediately after pyloric ligation[21]. Histamine (0.5mg/kg) was administered intra-peritoneally thirty minutes after extract administration. The animals were sacrificed 4 hours after the pyloric ligation. The stomachs were removed, contents collected, measured, centrifuged, and subjected to analysis for pH, and titrable acidity against 0.01N NaOH at pH7 with neutral red indicator[22]. The pH was measured using a digital pH meter (Consort P 107, Belgium). The protein content of the gastric juice was measured with the biuret method of Weichsebaum.

Statistical analysis

The collected data was reviewed, coded and analyzed using the Statistical Package for Social Sciences (SPSS) software version 17.0. The means and standard deviations were calculated and comparisons between treatments were done using Analysis of variance (ANOVA). The results were expressed as the mean, standard error of mean and differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical screening. Preliminary phytochemical test of the extract revealed the presence of tannins, flavonoids, glycosides, alkaloids, carbohydrate, protein, saponins and a neutral pH (7.0)

Acute toxicity test (Median Lethal dose, LD_{50}). The result of acute toxicity studies revealed that the methanolic extract of *M. paradisiacal* had an oral LD_{50} >5000 mg/kg in mice.

Cytoprotective effect and in combination with catecholamine. The results obtained from the study of the cytoprotective effect of the methanolic extract of *Musa paradisiaca* in combination with epinephrine and Dopamine offered a significant ($p<0.05$) ulcer inhibition in the glandular region of wistar albino rats. The ulcer index decreased from, 14.8 ±3.5 (ulcerated control) to 8.2 ± 1.4 and 4.8 ± 1.7 and gastric lesions inhibition of 44.60% and 67.57% in groups II and III respectively (Table 1). Administration of the extract alone showed significant ($p < 0.05$) ulcer inhibition with a decrease in the ulcer index from 14.8 ±3.5 (ulcerated control) to 3.0 ± 1.1 and gastric lesions inhibition of 79.73%. The result confirms that unripe plantain extract possess an anti-ulcerognic and cytoprotective effect on aspirin-induced gastric lesions.

It is possible that this cytoprotective action of unripe plantain (*M. paradisiaca*) is mediated by the action of endogenous prostaglandin which promotes mucus secretion and plays an important role in maintaining mucosal integrity against the actions of various damaging agents[23].Also the cytoprotective effects of the extract result from the enhancement of the mucosa barrier through the increased production of prostaglandins thereby antagonizing the actions of NSAIDS.

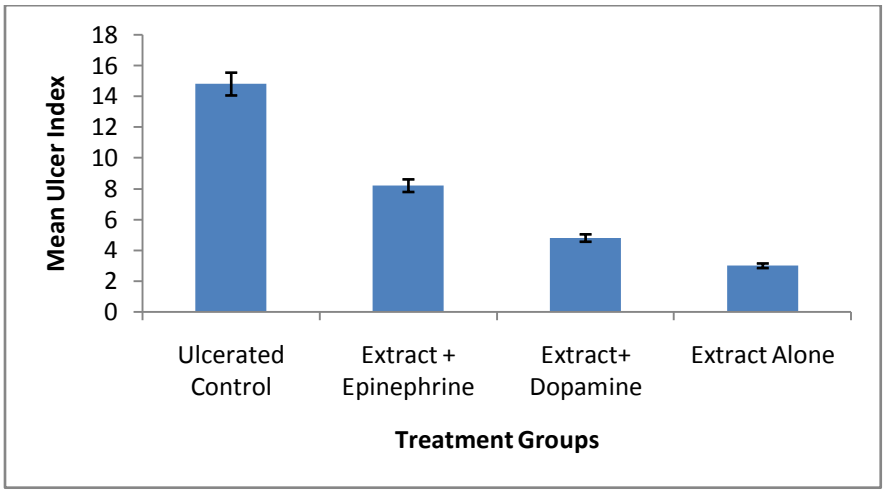

Figure 1: Bar chart showing the cytoprotective effects of *Musa paradisiaca* and in combination with catecholamines on indomethacin induced peptic ulcer. Result shown are mean ulcer index.

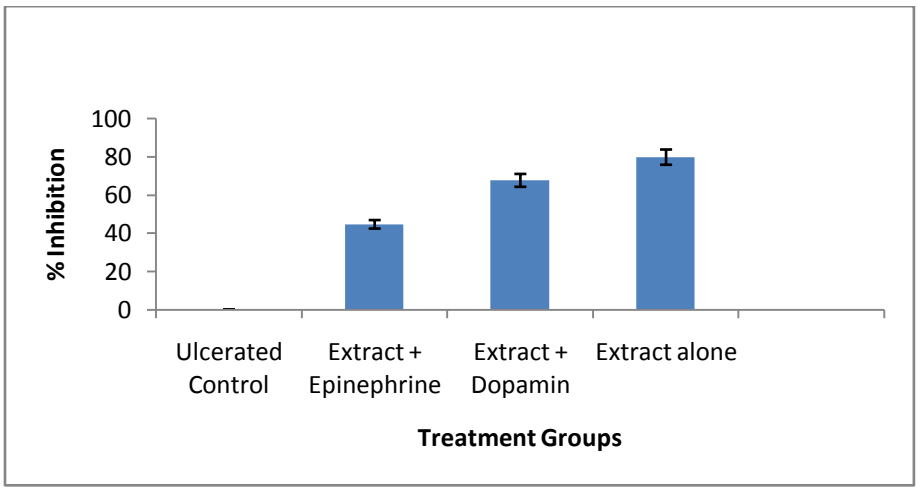

Figure 2: Bar chart showing the percentage inhibition of the methanolic extract of unripe plantain and in combination with catecholamines on indomethacin induced peptic ulceration in wistar albino rats.

The decrease in percentage inhibition effect of the extract in combination with the catecholamines, epinephrine and Dopamine in groups two and three respectively compared to percentage inhibition of the extract alone in group four could be as a result of the initial

activation of the sympathoadrenal system as was demonstrated by *Hans Selye* a Canadian physiologist in 1936. From Selye's hypothesis it has been revealed that the sympathoadrenal system becomes activated, with increased secretion of epinephrine and nor-epinephrine in response to stressors that challenge an organism to respond physically[24].Catecholamines are known to increase during physiological and psychological stress[25].

From available literature it has been revealed that under stressful conditions, there is increased ACTH (adrenocorticotrophic Hormones) release from the anterior pituitary and thus there is increased secretion of glucocorticoids from the adrenal cortex[24]. Glucocorticoids (a class of corticosteroids including cortisol) in turn exert permissive effects on the actions of catecholamines. And when these permissive effects are not produced because of abnormal low glucocorticoids, catecholamines will not be effective as they are normally .It is possible that increased secretion of glucocorticoids from the adrenal cortex stimulates the production of bleeding peptic ulcers.

It is possible that having subjected the rats to a 48 hours fast, the physiologic effects of hunger stress on the pituitary- adrenal axis coupled with the exogenous administration of catecholamine in experimental groups II and III exacerbated the indomethacin induced ulceration giving rise to complications that affected, invariably, the effects of the extract in combination with the catecholamine (Epinephrine and Dopamine). This collaborates with a study that showed that Dopamine and other catecholamines are involved in the pathogenesis of experimental duodenal and gastric ulcers in rats[26]. However, more research needs to be carried out in this regard to determine the direct mechanism of action of these hormones in combination with the extract.

Determination of anti-secretory activity. Treatment with *M. paradisiaca* extract resulted in a significant ($p<0.05$; Table 1) increase in gastric fluid (6.12±0.10 ml) after pyloric ligation and histamine stimulation compared with the negative control (1.76±0.20). The extract also significantly decreased titrable acidity of histamine treated rats (68.20±4.52mmol/L) when compared with negative control (206.4±7.81mmol/L). The extract (200 mg/kg body wt) significantly protected rats from ulceration after histamine administration in a four hour pyloric ligated rat with a protective index of 21.01±1.15 (82%) compared to that of the negative control (93.65±5.08). Cimetidine (H_2- receptor antagonist) protected pyloric-ligated rats significantly ($p<0.05$) from ulceration with an index of 7.26±1.10 (93.1%) and also made a significant increase in gastric pH (5.62±0.23), protein concentration in the gastric fluid (1398±73.51µg/100ml), and reduced the titrable acidity (39.4±6.50mmol/L) when compared with the negative control (Table 1).

Table 1 Effect of *M. paradisiaca* on gastric secretion, titable acidity, gastric pH, protein concentration of gastric fluid and ulceration in 4 hours pylorus ligated rats.

Group	Ulcer index	Vol of Gastric Juice (Ml)	Gastric pH	Protein Con.(µg/100ml)	Titrable acidity(mmol/L)
Saline	93.65±5.08	14.3±1.20	1.76±0.20	500±68.00	206.4±7.81
M.paradisiaca	21.01±1.15*	23.25±1.22*	6.12±0.10*	1165±62.01*	68.20±4.52*
Cimetidine	7.26±1.10*	25.30±1.18	5.62±0.23*	1398±73.51*	39.4±6.50*

**Significant at p<0.05*

The significant reduction of histamine induced gastric and inhition of ulcers by M. paradisiaca may involve direct reduction of gastric secretion through one or more possible mechanisms. It has been demonstrated that certain antiulcer drugs increase the amount of gastric mucous secretion in gastric mucosa[27]. The extract could also have exerted its effect by direct antagonism of H_2 – receptor stimulation by histamine through a process that is yet to be determined.

CONCLUSION

The results of the studies with the methanolic extract of *Musa paradisiaca* and in combination with catecholamines makes it a potent herbal drug for the treatment of peptic ulcer disease and prompts that chemistry of plantain pulp be studied more extensively to find out the active principle(s), which can be promising ulcer healing drug(s).Ulcer patients should take care to see that they are not exposed to catecholamine prescription before commencing (or during) treatment with *Musa paradisiacal* as the extract does not seem to show a favorable drug interaction with dopamine and epinephrine. Also, further studies should be made to see if this effect is true for all ulcer models including those caused by Helicobacter pylori.

RECOMMENDATION

It is apparent that experimental evaluation of herbal drugs for treatment of gastric ulcer is rather impressive, but very few have reached clinical trials and still few have been marketed. This shows that the benefits of research are not reaching the people to whom medical research is directed and hence the time, man power and resources are not efficiently utilized.

Hence, pharmacologists need to take more active interest in evaluation of herbal drugs for potential anti-ulcer activity and standardization of such herbal drugs to be clinically effective and globally competitive.

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