

## Effect of unripe *Musa paradisiaca* fruit on haematology, ulcer indices and antioxidant enzymes in rats induced with gastric ulcer

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### Abstract

**Background/Objectives:** Unripe plantain may have some medicinal properties which are hitherto unknown. This study investigated the haematological changes, oxidative status as well as the ulcer-healing potentials of unripe plantain (*Musa paradisiaca*) fruit in aspirin-induced gastric ulcer in the Wistar rat. **Materials and Methods:** Twenty-four (24) male rats were divided into six groups of four rats each. Group 1 served as normal control while ulceration was induced with oral administration of aspirin (225 mg/200 g body weight) in Groups 2 – 6 and subsequently treated with varying doses of (200 mg/kg, 400 mg/kg and 800 mg/kg body weight of rats) of the ethanol extract of *M. paradisiaca* and omeprazole 5 mg/kg body weight of rats for 5 days. **Results and Conclusion:** Induction of ulceration significantly ( $P < 0.05$ ) increased the apparent body weight of the rats, which was not reversed by treatment with graded doses of ethanol extract of *M. paradisiaca*. There were no significant changes ( $P > 0.05$ ) in platelets, lymphocytes, neutrophil, eosinophil, basophil, red blood cell, hemoglobin and hematocrit concentrations in all of the test groups when compared with the control. Administration of graded doses of *M. paradisiaca* ethanol extract reversed the activities of SOD and GPx to the normal control. Treatment with the extract also produced a non-significant ( $P > 0.05$ ) increase in the MDA which was comparable with the positive control. There was a significant ( $P < 0.05$ ), dose-dependent-decrease in the ulcer index in the groups administered varying doses of the extract compared with the untreated control, with the group administered 800 mg/kg B. wt. of extract presenting a mean value of  $0.667 \pm 0.33$ , which was more effective than the standard drug ( $2.33 \pm 0.88$ ). Furthermore, the group that was administered the highest dose of the ethanol extract showed the highest percentage ulcer inhibition (95.45 %) compared with the positive control (79.10 %). This research showed that the ethanol extract of unripe *Musa paradisiaca* fruit possesses ulcer-healing potential and should be recommended as an ideal diet for individuals on high-risk category of gastric ulcer resulting from non-steroidal anti-inflammatory drugs (NSAIDs) treatment.

**Keywords:** *Musa paradisiaca*; ulcer; haematology; antioxidant, protection index

### Introduction

An ulcer is an open sore in the mucous membranes of the gastrointestinal tract. It affects a large percentage of people in the world. An ulcer can be caused by several factors, the most common being an infection caused by *Helicobacter pylori*. The bacteria damage the protective mucous layer of the stomach, which allows stomach acid to enter the stomach lining. Other factors that can

result in ulceration include taking certain painkillers (aspirin, etc.), smoking and excessive consumption of alcohol. Stress and anxiety are also factors that can lead to ulcer formation (1). Traditional ulcer treatments consist of acid-suppressant drugs and antibiotics. Avoiding risk factors like smoking and binge drinking, controlling stress and using NSAIDs with caution are all part of prevention methods (2). Aspirin is an

acetylsalicylic acid often used to treat pain, fever and inflammation. Despite its therapeutics benefits, it is known to induce gastric ulcer in both human and animals (3). However, the increase in antibiotic resistance and the side effects associated with medication use have led to an increase in the demand for alternative therapies such as medicinal plants, *M. paradisiaca* (plantain) inclusive (4).

The treatment and control of disorders like ulcers have substantially benefited from medicinal herbs. Plantain is a plant species of the plantago family that has a long history of being used as a natural remedy (5). The Musaceae family's *M. paradisiaca* is a therapeutic plant that is known to have a variety of biological effects, including anthelmintic, anticancer and hypoglycemic (6). Therefore, the purpose of this study is to evaluate the ulcer-healing ability of the ethanol extract of unripe *M. paradisiaca* fruit, and to assess the antioxidant and haematological changes that occur as a result of treatment (7).

## Materials and methods

### Plant Materials

Unripe *M. paradisiaca* fruit was obtained from New Benin Market, Benin City, Edo State, Nigeria. The fruit was washed, peeled, air-dried and ground into powered form from which 2000 g was soaked in 5 liters of ethanol for 72 hours with periodic stirring of the mixture. Thereafter, the mixtures were filtered with clean sieve cloth, the filtrate was further filtered with Whatman filter paper (No. 1) and subsequently concentrated with the aid of a vacuum concentrator at 30°C. The extracts were subsequently freeze-dried at the National Centre for Energy and Environment, University of Benin, Benin City, Edo State, and stored in a refrigerator until required for analysis.

### Animal

Twenty-eight (28) Wistar rats weighing 140 – 250 g were purchased from the Animal House of Anatomy Department, University of Benin, Benin City, Edo State. They were maintained according to the Institutional Animal

### Study

Guidelines

(1912/PO/Re/S/16/CPCSEA) and acclimatized to diet and environment for 2 weeks after arrival. They were housed in a density of four animals per rack mounted cages and were given clean drinking water and Chikun growers feed. The temperature (20 – 22 °C) and light (12-hour light/dark cycle) were constantly controlled. The rats were randomly distributed into six groups as follows:

Group 1 – Normal control (no induction; administered 2 mL/kg of distilled water)

Group 2 – Negative control (ulcer induction, no treatment).

Group 3 – Ulcer induction, administered 800 mg ethanol extract of *M. paradisiaca* per kg B. wt. of rat

Group 4 – – Ulcer induction, administered 400 mg ethanol extract of *M. paradisiaca* per kg B. wt. of rat

Group 5 – Ulcer induction, administered 200 mg ethanol extract of *M. paradisiaca* per kg B. wt. of rat

Group 6 – Ulcer induction, administered Omeprazole (5 mg/kg B. wt. of rat).

### Induction of Ulcer

Aspirin was purchased from a pharmaceutical shop in the University of Benin, Nigeria. Animals from all groups were starved for 48 hours after which 225 mg/200 g of body weight single dose of aspirin was administered to all the groups of rats excluding group 1.

### Animal Sacrifice/Sample Collection

The animals were handled according to the Guidelines for the Treatment of Laboratory Animals. They were sacrificed at the end of the five (5) days administration period i.e., on the sixth day, after an overnight fast. Each rat was anaesthetized in a Chloroform (CHCl<sub>3</sub>) saturated container and blood sample collected via a heart puncture into labelled Lithium Heparin and Ethylene Diamine Tetra-Acetic Acid (EDTA) tubes.

### Determination of the ulcer index and percentage inhibition of ulcer

To determine the ulcer scores, the stomach was incised along the area of greater curvature and the wall was viewed using a hand lens (×5). The ulcerative index was

counted and scored as follows: Normal stomach = 0; Pin hole = 1.0; Spot ulceration = 1.5; Haemorrhagic streaks = 2.0; Small erosion = 2.5; Large erosion = 3.0; Perforation = 3.5. The mean ulcer scores for each animal were expressed as ulcer index. The percentage inhibition (protection index) was calculated using;

$$\% \text{ Inhibition} = (1 - (\text{ulcer index with extract} \div \text{ulcer index with distilled water})) \times 100 \% \text{ (Reference)}$$

#### Biochemical assays

##### Estimation of antioxidant parameters

Assay for some antioxidant enzymes were performed according to established protocols. Superoxide dismutase (SOD) level was estimated using the method (8), while the glutathione peroxidase (GPx) levels was determined by [9] and the assay method (10) modified by (11) was used for the estimation of malondialdehyde content.

##### Determination of haematological parameters

Haematological parameters were determined using an Automatic Haematology Analyzer (Sysmex, Canada).

##### Statistical analyses

Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean  $\pm$  standard error of mean (SEM). Differences between the mean values between groups were compared by the Fisher's Least Significant Difference (LSD) post hoc test-, and  $P$  values  $< 0.05$  were considered statistically significant.

## Results and discussion

### Comparison of the relative body weight in ulcerated rats

The effect of the ethanol extract of unripe *M. paradisiaca* on the weight of the liver as a function of the body weight is presented in Table 1. In comparison with the control, the ratio was seen to increase in all groups with a significant increase observed in the groups administered the highest dose of the extract as well as the group given the standard drug.

### Effect of *M. paradisiaca* extract on the ulcer index of aspirin-induced ulcerated rats

Table 2 highlights the effect of the unripe fruit extract of *M. paradisiaca* on the mean ulcer index in the aspirin-induced ulcerated rats. The result shows an ulcer index of  $11.00 \pm 0.57$  in the untreated group. There was a significant ( $P < 0.05$ ), dose-dependent-decrease in the ulcer index in the groups administered varying doses of the extract compared with the untreated group, with the group administered 800 mg/kg B. wt. of extract presenting a mean value of  $0.667 \pm 0.33$ , which was more effective than the standard drug ( $2.33 \pm 0.88$ ). Furthermore, the group that was administered the highest dose of the ethanol extract showed the highest percentage ulcer inhibition (95.45 %) compared with the positive control (79.10 %).

### Effect of *M. paradisiaca* extract on the antioxidant levels in aspirin-induced ulceration

Compared to the control group, there was a significant decrease ( $P < 0.05$ ) in the superoxide dismutase (SOD) levels in the group with untreated ulcer. The groups administered 800 and 400 mg/kg B. wt. of the ethanol extract reversed the SOD to similar levels as the normal control. The malondialdehyde concentration in the untreated ulcerated group was also found to decrease significantly ( $P < 0.05$ ) in comparison with the normal control. However, treatment with ethanol extract of *M. paradisiaca* produced a non-significant ( $P > 0.05$ ) increase in the MDA albeit comparable with the group treated with Omeprazole. Furthermore, the glutathione peroxidase concentration was significantly ( $P < 0.05$ ) decreased in the group 2 rats compared to group 1. This decrease was ameliorated when treated with graded doses of the ethanol extract of unripe plantain fruits.

### Effect of *M. paradisiaca* extract on some haematological parameters in aspirin-induced ulceration

The haematological parameters of the rats treated with ethanol extract of *M. paradisiaca* are compared with the control in Table 4. There was no significant difference ( $P > 0.05$ ) in the white blood cells (WBC), red blood cells (RBC) and platelets (PLT) in the untreated ulcer group compared to the normal control. These parameters also remain unchanged in the groups administered graded doses of the ethanol extract of *M. paradisiaca*.

### Discussion

Medicinal plants have continued to attract attention in the global search for effective antimicrobial agents that can combat resistant pathogens that have been rendering many conventional drugs recalcitrant in the treatment of infections. Plants are the most exclusive sources of drugs for the majority of the world as people in the developing countries use medicinal plants for their primary health care (12). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and acute diseases and both traditional healers and pharmaceutical drug companies exploit these attributes (13). This study examined the effect of ethanol fruit extract of unripe *Musa paradisiaca* on some hematological parameters, relative body weight, mean ulcer index and percentage ulcer inhibition in aspirin-induced gastric ulceration in male Wistar rat.

Determination of the organ weight to body ratio (known as the relative organ weight) is widely accepted as an indication of the toxicity of a treatment or chemical entity (14). The result show that induction of ulceration with aspirin caused a significant increase in the liver to body weight ratio when compared to the normal control, indicating that administration of 225 mg/200 g of body weight single dose of aspirin was toxic to the liver. The decrease in the

absolute weight and an increase in the relative weight of the organ are consistent with the report (15). Treatment with graded doses of the ethanol extract of *M. paradisiaca* did not reverse the aspirin-induced relative organ change in the rats. Administration of the standard drug was proficient in reversing the aspirin-induced toxicity which was not the case with the ethanol extract of the unripe plantain fruits. Previous reports have shown that administration of fermented unripe *M. paradisiaca* fruit [16], plantain peel extract of unripe plantain ([17,18) and ethanol leaf extract (19) reduces the ulcer index. These reports are in agreement with the result of this study wherein the extract showed a significant reduction in the mean ulcer index in the groups treated with 800 mg/kg, 400 mg/kg and 200 mg/kg B. wt. of ethanol fruit extract of unripe *M. paradisiaca* when compared to the untreated group with the group administered 800 mg/kg B. wt. of extract presenting a more effective index than the standard drug. Furthermore, the group that was administered the highest dose of the ethanol extract showed the highest percentage ulcer inhibition (95.45 %) compared with the positive control (79.10 %). This is consistent with the finding of (20) who reported that *M. paradisiaca* pulp extract exhibited considerable ulcer inhibition in indomethacin-induced ulceration in Wistar rats.

The remarkable anti-ulcer activity and cytoprotective properties of the ethanol extract of unripe plantain (*Musa paradisiaca*) on the experimental rat may be attributed to its phytochemical constituents, flavonoids, tannins, and saponins (21).

Cellular metabolism generates reactive oxygen species (ROS) such as superoxide radical ( $O_2^-$ ) and hydroxyl radical ( $OH^-$ ). Oxidative stress, present in the process of gastric ulceration, increases the formation of reactive oxygen species (ROS) that can disrupt epithelial cell integrity. An increased production of ROS metabolites may overwhelm the endogenous antioxidants

(22). Superoxide dismutase (SOD) catalyzes the breakdown of superoxide anion free radical ( $O_2^-$ ) into molecular oxygen and hydrogen peroxide ( $H_2O_2$ ), which is subsequently converted into water by catalase and peroxidases. In this study, there was a significant decrease ( $p < 0.05$ ) in the superoxide dismutase (SOD) activity in the aspirin-induced ulcer group. Increase in the SOD activity correlates with an increased production of ROS. The groups administered 800 and 400 mg/kg B. wt. of the ethanol extract reversed the SOD to similar levels as the normal control, indicating that the extract was proficient in reducing the ROS. In addition, the malondialdehyde levels, which correlates with the level of lipid peroxidation in the cell, was found to decrease significantly ( $p < 0.05$ ) in the untreated ulcerated group compared with the normal control. However, treatment with ethanol extract of unripe plantain fruits produced a non-significant ( $p > 0.05$ ) increase in the MDA which was comparable with the group treated with Omeprazole. Furthermore, the glutathione peroxidase activity was significantly ( $p < 0.05$ ) decreased in the group 2 rats compared to group 1. The decrease was ameliorated with the administration of graded doses of the ethanol extract. Increases in malondialdehyde level is commonly considered as an indicator for lipid peroxidation derived from oxidation stress,

therefore from this result, there is a decrease in malondialdehyde level which indicates an absence of lipid peroxidation in the liver. Finally, the result of this study showed no significant difference in the hematological parameters between the normal control rats and the untreated ulcerated rats. The concentration of WBCs including granulocytes, monocytes and basophils as well as RBCs and platelets levels did not differ significantly between the treatment groups and the untreated group. Previous report by (23) showed that in rats treated with stem extracts of *M. paradisiaca*, the WBC count was found to increase, an opposite finding in this research. There was no significant change ( $P > 0.05$ ) in platelet count in all the test groups when compared with the control. This non-significant change in platelet count is consistent with the finding of (24) who reported that the methanol extract of ripe fruit peels of *M. paradisiaca* led to a no-significant change in platelet count.

### Conclusion

The ethanol fruit extract of unripe *Musa paradisiaca* is efficient in the treatment of gastric ulcer as it reduces the mean ulcer index and showed remarkable percentage ulcer inhibition scores in aspirin-induced gastric ulceration in male Wistar rats in a dose-dependent manner. Furthermore, administration of the extract did not alter the haematological parameters.

**Table 1: Effect of ethanol fruit extract of unripe *M. paradisiaca* on liver/body weight ratio in male Wistar rat.**

Groups	Liver/Body weight ratio ( $\times 10^{-2}$ )
Group1(Control)	2.73 $\pm$ 0.13
Group 2 (Untreated)	3.68 $\pm$ 0.45*
Group 3 (800 mg/kg B. wt. of extract)	3.73 $\pm$ 0.26*
Group 4 (400 mg/kg B. wt. of extract)	3.16 $\pm$ 0.08
Group 5 (200 mg/kg B. wt. of extract)	3.22 $\pm$ 0.29
Group 6 (5 mg/kg B. wt. of Omeprazole)	2.49 $\pm$ 0.13#

Data reported as Mean  $\pm$  Standard Error of Mean, n = 3. \* -  $P < 0.05$  compared to the control; # -  $P < 0.05$  compared to the untreated group,

**Table 2: Effect of ethanol fruit extract of unripe *M. paradisiaca* on mean ulcer index and percentage ulcer inhibition in aspirin-induced peptic ulcer in male Wistar rat**

Groups	Mean ulcer score	Ulcer index	% inhibition
Group1(Control)	-	-	-
Group 2 (Untreated)	2.00±0.40	11.00±0.57	-
Group 3 (800 mg/kg B. wt. of extract)	1.00±0.00 <sup>#</sup>	0.667±0.33 <sup>#</sup>	95.45
Group 4 (400 mg/kg B. wt. of extract)	1.14±0.14 <sup>#</sup>	3.33±0.33 <sup>#</sup>	72.70
Group 5 (200 mg/kg B. wt. of extract)	1.40±0.18 <sup>#</sup>	5.66±0.33 <sup>#</sup>	48.19
Group 6 (5 mg/kg B. wt. of Omeprazole)	1.50±0.29 <sup>#</sup>	2.33±0.88 <sup>#</sup>	79.10

Data reported as Mean ± SEM, n = 4. \*  $P < 0.05$  compared to the control; #  $P < 0.05$  compared to the untreated group.

**Table 3: Effect of ethanol fruit extract of unripe *M. paradisiaca* on liver SOD, MDA and GPx in aspirin-induced gastric ulceration in male Wistar rat**

Groups	SOD	MDA	GPx
Group1(Control)	0.74±0.03	0.58±0.08	1.80±0.08
Group 2 (Untreated)	0.61±0.05 <sup>*</sup>	0.17±0.01 <sup>*</sup>	1.49±0.10 <sup>*</sup>
Group 3 (800 mg/kg B. wt. of extract)	0.75±0.02 <sup>#</sup>	0.20±0.01 <sup>*</sup>	1.93±0.04 <sup>#</sup>
Group 4 (400 mg/kg B. wt. of extract)	0.75±0.03 <sup>#</sup>	0.20±0.02 <sup>*</sup>	1.81±0.08 <sup>#</sup>
Group 5 (200 mg/kg B. wt. of extract)	0.67±0.03	0.23±0.01 <sup>*#</sup>	1.56±0.08
Group 6 (5 mg/kg B. wt. of Omeprazole)	0.58±0.08 <sup>*</sup>	0.22±0.02 <sup>*#</sup>	1.39±0.11 <sup>*</sup>

Data reported as Mean ± Standard Error of Mean, n = 3. \*  $P < 0.05$  compared to the control; #  $P < 0.05$  compared to the untreated control

**Table 4: Effect of ethanol fruit extract of unripe *M. paradisiaca* on haematological parameters in aspirin-induced gastric ulceration in male Wistar rats.**

Groups	WBC (x10 <sup>3</sup> )	LYM (%)	MON (%)	NEU (%)	EOS (%)	BAS (%)	RBC(x10 <sup>6</sup> )	HGB (g/L)	HCT (%)	PLT (x10 <sup>3</sup> )
Group1(Control)	10.53±1.14	84.47±1.73	3.07±0.66	9.77±1.47	0.60±0.15	5.57±0.70	8.82±0.32	14.67±0.09	48.80±0.64	677.33±13.28
Group 2 (Untreated)	13.23±0.64	82.23±2.30	5.53±1.85	7.27±1.84	0.27±0.03	5.70±1.59	8.43±0.53	14.00±0.67	44.40±1.85	550.33±24.74
Group 3 (800 mg/kg B. wt. of extract)	12.13±1.33	86.50±1.26	3.10±0.49	6.80±0.44	0.60±0.21	3.63±0.48	8.55±0.33	15.67±0.84	47.90±0.90	579.33±15.34
Group 4 (400 mg/kg B. wt. of extract)	14.13±1.27	87.73±2.23	6.43±1.39	6.73±2.99	0.47±0.12	2.10±0.61	9.14±0.62	15.73±0.69	51.60±1.99 <sup>#</sup>	662.67±57.24
Group 5 (200 mg/kg B. wt. of extract)	11.23±1.09	82.37±5.26	6.73±1.73	4.43±1.39	0.90±0.55	5.57±2.64	9.15±0.93	14.83±0.84	48.60±3.02	611.67±88.89
Group 6 (5 mg/kg B. wt. of Omeprazole)	17.03±3.00 <sup>*</sup>	78.57±2.15	13.27±6.27 <sup>*</sup>	8.30±1.77	0.43±0.07	8.13±0.54	9.32±0.35	15.50±0.59	49.00±1.76	542.67±30.89

Data reported as Mean ± Standard Error of Mean, n = 3. \*  $P < 0.05$  compared to the control; #  $P < 0.05$  compared to the untreated control

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