Trop J Nat Prod Res, January 2024; 8(1):6035-6038 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of Proliferative Potential of *Musa Paradisiaca* Stem Juice on the Pancreatic Cells of Wistar Rats

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ARTICLE INFO

Article history:
Received 31 October 2023
Revised 01 January 2024
Accepted 11 January 2024
Published online 01 February 2024

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ABSTRACT

Musa paradisiaca commonly called plantain is a major part of human diet especially in West Africa. It is used as the main source of energy food for people with type 2 diabetes mellitus. The purpose of this study was to assess the ability of *Musa paradisiaca* stem juice to boost pancreatic cell proliferation in Wistar rats. Twenty-four adult Wistar rats were divided into four groups of six animals each. The first group (control) was administered normal saline, while groups 2 - 4 were administered 10, 20, and 30 mL/kg orally of plantain stem juice, respectively once daily for 21 days. The effect of plantain stem juice on body weight and haematological indices were evaluated. The cell proliferative and/or cytotoxic potential of the juice was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay. The effect on interleukin-4 was also evaluated using the enzyme-linked immunosorbent assay (ELISA). Administration of plantain juice resulted in a significant increase in the body weight of the rats. There was no significant effect on the haematological parameters except for an increase in white blood cell count. Plantain stem juice tends to increase pancreatic cell proliferation and improves the histomorphology of the pancreas after 21 days of administration. Therefore, *Musa paradisiaca* stem juice may serve as a protective agent against pancreatic cell damage or dysfunction.

***Keywords*:** Pancreas, Plantain, Haematology, Cytotoxicity, MTT

Introduction

The pancreas is an organ measuring 6 - 10 inches in length situated in the upper left abdominal segment behind the stomach and encircled by the small intestine, liver, and spleen.[1] The pancreas stretches horizontally over the belly, it is a flat spongy organ with a pear-like or fish-like appearance. The three parts of the pancreas are the head, which is located at the junction of the stomach and duodenum; the center portion, also referred to as the neck or body; and the tail, which reaches to the left side of the abdomen.[2,3] The pancreas is surrounded by major blood vessels that supply it and other abdominal organs, such as the celiac axis, portal vein, superior mesenteric artery, and superior mesenteric vein.[4] The pancreas play a major role in digestion and regulation of blood sugar. It secretes pancreatic juice which contain digestive enzymes like lipase, protease, and amylase. It also produces hormones like insulin and glucagon that help regulate blood sugar.

For many people, especially in the developing nations, traditional medicine especially medicinal plants are the main source of treatment for a variety of illnesses. Thes plants are of several varieties, including trees, shrubs, woody plants, climbers, annuals, biannuals, or perennials.5,6 Musa paradisiaca juice has been used in traditional medicine to treat a variety of illnesses, including inflammation, fever, indigestion, and constipation.[7-9] The objective of this study was to evaluate the proliferative potential vis-à-vis the protective effect of Musa paradisiaca on the pancreas of Wistar rats.

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Citation: Oyepata JS and Opeyemi JT. Evaluation of Proliferative Potential of *Musa Paradisiaca* Stem Juice on the Pancreatic Cells of Wistar Rats. Trop J Nat Prod Res. 2024; 8(1):6035-6038. http://www.doi.org/10.26538/tjnpr/v8i1.46

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Materials and Methods

Musa paradisiaca stems were purchased at Itam Market in Uyo, Akwa Ibom State on May 18, 2022. The plant material was identified and authenticated by Mr Udom Nisikan at the Department of Botany, University of Uyo, Akwa Ibom State, Nigeria. Herbarium specimen with voucher number UY811 was deposited at the herbarium of the department.

Plant extraction

About 3kg of fresh stem of *Musa paradisiaca* were bought from Itam Market in Uyo, Akwa Ibom State. They were chopped into small pieces, pounded with mortar and pestle and juice squeezed out. The juice was extracted from the resulting mash using a press. The extracted juice was kept in a clean container. It is from this container that the juice was regularly measured out for the animals

Animals

Twenty-four (24) adult Wistar rats (196 – 216g) of either sex were obtained from the Nigeria Derindam Research Institute of Biotechnology, Uyo, Akwa Ibom State. The rats were housed in rodent cages in the animal housing facility of the research institute. The rats were fed with normal rodent pellet and had access to drinking water *ad libitum*. The animals were kept under regular environmental conditions, and handled according to the international guidelines for the use and care for experimental animals. The Animal Ethics Committee of University of Uyo College of Health Sciences issued authorization and approval for animal studies (UY/2022/1161).

Study Design

Twenty-four adult Wistar rats were divided into four groups of six rats per group. Group 1 served as the control group and was administered distilled water. Groups 2, 3, and 4 were administered 10, 20, and 30 mL/kg of *Musa paradisiaca* (plantain) stem juice, respectively. The administration was done orally, once daily for twenty-one days. The

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body weight of the rats was monitored throughout the period of administration. After twenty-one days of administration, the rats were sacrificed, and blood samples were collected by cardiac puncture for hematological analysis. The pancreas was harvested and prepared for immunoassay and histological investigations.

Haematological analysis
The blood samples were placed in EDTA bottles until ready for use. The following haematological parameters were evaluated; white blood cell counts (WBC) and differentials (neutrophils, eosinophils, basophils, lymphocytes, and monocytes), red blood cell counts (RBC), haemoglobin (Hb) concentration, and platelets count. The analysis was done using an automated haematology machine (Cell-Dyn, Abbott, USA) following standard procedure.[10]

Histological investigation
The harvested pancreas was preserved for 24 hours in 10% neutral-buffered formalin, and then the tissue was fixed in 10% neutral-buffered formalin for forty minutes, and then sectioned for histological examination[11].

Enzyme Linked Immunosorbent Assay for Interleukin-4
This assay is based on an antibody-antigen response which is produced upon the addition to a cell culture of a goat polyclonal anti-mouse IL-4 detection antibody that has been biotinylated. Addition of Avidin-Biotin-Peroxidase complex (ABC) followed by the peroxidase substrate TMB solution induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of IL-4 present in the samples.
Briefly, standard concentration of interleukin-4 antibodies and were captured in a coating buffer. The captured antibodies (50 µL) were added to each well in a 96-well strip plates pre-coated with monoclonal anti-rat IL-4 antibody, and incubated for 60 min. The coating buffer (5 mL) was added into a trough and mixed properly, 50 µL of the diluted antibody was transferred into each microwell containing the test samples. The microwell and its contents were incubated for 60 min to allow complexation with the bound IL-4 antibody. A biotinylated anti-rat IL-4 antibody is then added. After incubation, the medium was aspirated and discarded, each well was washed 3 times using the washing solution followed by the addition of 100 µL of an enzyme Avidin-Biotin-Peroxidase complex which binds to the second antibody. The peroxidase substrate TMB was then added to induce a coloured reaction product.[8]

Primary cell isolation
The Pancreas was dissected, and any unusable tissue was removed. The remaining tissue was minced into pieces of 3 to 4 mm each using a sterile scalpel or scissors. The tissue pieces were washed and suspended in a modified balanced salt solution without calcium and magnesium. This process was repeated two to three times, and the container with the tissue pieces was placed on an ice bath. Any leftover supernatant was decanted, and 0.25% trypsin was added to the balanced salt solution without calcium or magnesium. Two equal portions of the separated cells were placed in microtubes A and B, respectively.[9]

Cell Proliferation and Cytotoxicity Assay
The harvested cells were resuspended in cell isolating fluid and centrifuged and the supernatant was discarded. A serial dilution of cells in culture medium were prepared and 50 µL of the diluted cells were transferred into the wells of microtiter plate and incubated at 37°C for 12 hours, after which 20 µL of Molecular Targeted Therapies reagent was added to each well including controls. Thereafter, it was incubated at 37°C for another 2 hours, after which 50 µL of MTT reagent were added to all the wells including the controls and swirled gently. The plate was incubated at room temperature for 3 hours, and then placed on the microtiter plate reader and the optical density was measured at a wavelength range of 550-660 nm.[9]

Cell Viability Assay
The separated cells were centrifuged and the supernatant was removed. Trypan blue solution (100 mL) was added to 50 mL of the cell pellets. The cells were then placed in the neuber chamber, and the viable cells were counted.[10]

Statistical Analysis
Data were presented as mean ± standard error mean. The data were analyzed by one-way analysis of variance. P-value of less than 0.05 ($p < 0.05$) was regarded as statistically significant.

Result and Discussion

Effects of Musa paradisiaca stem juice *on body weight*
It was observed that the administration of *Musa paradisiaca* stem juice resulted in an increase in the body weight of the rats used in this study. Rats in the treatment groups (2, 3, and 4) showed significant increase in their body weight compared to the control group (Figure 1). The increase in body weight could be attributed to the phytonutrients such as fats, carbohydrates, vitamins, minerals, and other phytochemicals present in the plantain stem juice. Plantain has the potential to become an important part of both human and animal diets because of its high nutritional content in lipids, carbohydrates, vitamins, and minerals.[11] Plantain is an excellent nutritional source in diabetic patients because of its potential in maintaining blood sugar level. Additionally, plantain is a rich source of phytochemicals with antioxidant properties. The antioxidant action of these phytonutrients could contribute to their ability in maintaining blood sugar levels.[12] It has been observed that *Musa paradisiaca* stem juice has the ability to regulate body weight depending on the health status of an individual, for example, it could cause weight loss in sick individuals and weight increase in healthy individuals.[13]

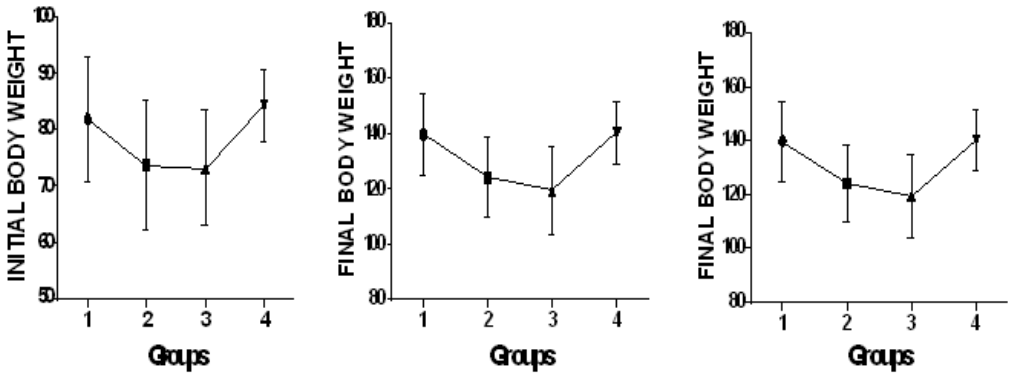

Figure 1: Effect of *Musa paradisiaca* stem juice on body and organ weight of rats

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Effects of Musa paradisiaca stem juice on haematological parameters
The investigation of the effect of *Musa paradisiaca* stem juice on the hematological parameters in rats showed that the administration of the juice resulted in a significant elevation of white blood cell count, while red blood cell count, packed cell volume, and platelet count were not significantly affected (Table 1). In addition, no significant effect on white blood cell differentials was observed (Table 2). This observation suggests that *Musa paradisiaca* stem juice is not haematotoxic, do not suppress the hemopoietic system, and could help boost the immune system as a result of its ability to increase white blood cell count.

Effects of Musa paradisiaca stem juice on interleukin-4 (IL-4)
At a dose of 10 mL/kg of *Musa paradisiaca* stem juice, there was no significant effect on the level of interleukin-4 (IL-4) compared to the control group. Whereas at 20 and 30 mL/kg, there was a significant increase in the IL-4 level between the treatment group and the control group (Table 3).

Cell proliferative and cytotoxic effects of Musa paradisiaca stem juice
Administration of plantain stem juice did not cause any cytotoxic effect on the pancreatic cells of the rats at the doses tested. In comparison to the control group, there was an increase in pancreatic cell proliferation in the treatment groups (Table 4). This observation suggests that plantain juice may have no effect on key growth regulatory factors, and other cytokines that are crucial in the cell cycle progression. As a result, cell division, proliferation, and the equilibrium between cell division and cell death are maintained in favour of cell proliferation. The cell proliferative potential of plantain stem juice may be attributed to the phytochemical constituents of the juice. The MTT assay is a fast and simple quantitative measure of cell growth and viability.[14] it is based on the ability of mitochondrial dehydrogenase in viable cells to reduce the yellow MTT solution to a purple formazan crystals whose absorbance can be measured spectrophotometrically at 550 – 660 nm.

Effects of Musa paradisiaca stem juice on pancreatic histomorphology
The importance of the pancreas as an endocrine and exocrine gland cannot be overemphasized. The production of insulin which help regulates blood sugar, metabolism, and the secretion of digestive enzymes are among the main functions of the pancreas.[15,16] The microscopic appearance of this organ has proven to be very helpful in medical diagnosis. The pancreatic architecture, cellular profile, serous gland, interstitium, islet cells of Langerhans, alpha and beta cells, and blood vessels within the normal cellular environment were all intact in the juice-treated groups (Figure 2). These findings are in line the studies that suggest the stem juice of *Musa paradisiaca* is generally safe in low to moderate doses. It is important to note that the study supports the idea that plantain stem juice has a favorable influence on pancreatic histomorphology, and as a result may serve as a protective agent against pancreatic cell damage or used to ameliorate pancreatic cell dysfunction.

Table 1: Effect of *Musa paradisiaca* stem juice on haematological parameters of Wistar rats

Group	PCV (%)	RBC (x10^6/µL)	WBC (/µL)	Platelet (x10^5/µL)
Control (N/S)	38.17 ± 3.70	4.30 ± 0.61	5434.00 ± 18.01	4.500 ± 1.565
M.P 10 mL/kg	45.17 ± 0.87	4.10 ± 0.60	7500.00 ± 75.32*	2.667 ± 0.670*
M.P 20 mL/kg	44.50 ± 1.84	3.30 ± 0.45*	7731.00 ± 24.99*	2.667 ± 0.670*
M.P 30 mL/kg	45.00 ± 0.00	3.87 ± 0.82	9598.00 ± 24.45*	3.167 ± 0.870*

Values are Mean ± SEM (n = 6). M.P = *Musa paradisiaca*, PCV = Packed cell volume, RBC = Red blood cell count, WBC = White blood cell count
.

Table 2: Effect of *Musa paradisiaca* stem juice on white blood cell differentials

Group	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)
N/S+feed only	14.33 ± 2.39	48.00 ± 16.27	1.83 ± 0.65	0.33 ± 0.21	3.50 ± 0.88
M.P 10 mL/kg	11.00 ± 2.54	51.50 ± 7.27	3.67 ± 1.23*	0.50 ± 0.22	4.00 ± 1.16
M.P 20 mL/kg	9.50 ± 1.91	54.33 ± 13.74	0.83 ± 0.17	0.17 ± 0.17	3.00 ± 0.73
M.P 30 mL/kg	14.00 ± 2.92	38.67 ± 4.90*	2.17 ± 0.65	0.50 ± 0.23	4.00 ± 0.77

Values are Mean ± SEM (n = 6). *significantly different compared to control ($p < 0.05$); M.P = *Musa paradisiaca*

Table 3: Effect of *Musa paradisiaca* stem juice on Interleukin-4

Group	Interleukin-4 (%)	MTT (%)
N/S + feed only (control)	0.91 ± 0.29	3.16 ± 1.08
M.P 10 mL/kg	1.43 ± 0.05	3.97 ± 0.52
M.P 20 mL/kg	1.52 ± 0.05[a]	4.17 ± 0.49[a]
M.P 30 mL/kg	1.51 ± 0.11[a]	3.10 ± 0.26

Values are mean ± SEM (n = 6). [a] = significant difference ($P < 0.05$). M.P = *Musa paradisiaca*

Conclusion

The findings from this study have shown that administration of plantain stem juice to rats at a low to moderate doses is relatively safe. No negative effect on haematological parameters, and pancreatic cell viability was observed. In addition, the study revealed that administration of *Musa paradisiaca* stem juice improves the histomorphology of the pancreas, and as a result may ameliorate pancreatic dysfunction.

Conflict of Interest

The authors declare no conflict of interest.

Table 4: Effect of *Musa paradisiaca* stem juice on cell viability

Group	Dead Cells (%)	Live Cells (%)
N/S + feed only (control)	7.75 ± 1.109	4.75 ± 0.85
M.P 10 mL/kg	4.70 ± 1.250*	5.750 ± 1.70*
M.P 20 mL/kg	4.50 ± 0.65*	2.75 ± 1.75*
M.P 30 mL/kg	5.25 ± 1.60*	3.25 ± 0.95*

Values are Mean ± SEM (n = 6). * = significantly different compared to control group ($p<0.05$). M.P = *Musa paradisiaca*

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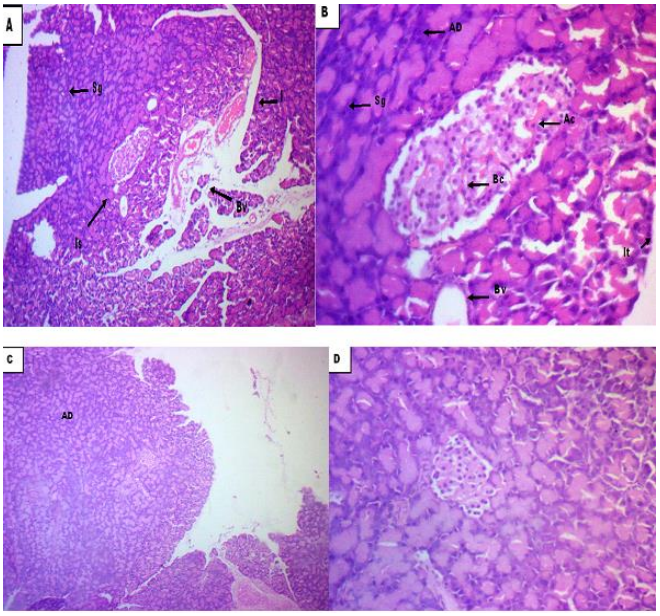

Figure 2: Photomicrograph of pancreatic cells of rats. (**A):** normal saline, (**B):** Pancreas treated with 10 mL/kg of *Musa paradisiaca* stem juice, (**C):** Pancreas treated with 20 mL/kg of *Musa paradisiaca* stem juice and (**D):** Pancreas treated with 30 mL/kg of *Musa paradisiaca* stem juice. Magnification; C(X100) and D(X400), stained with H&E.
Key: Acini Duct (**AD**), Serous gland (**Sg**), Interstitium (**It**), Islet cells of Langerhans (**Is**), Alpha cells (**Ac**), Beta cells (**Bc**), Blood vessel (**Bv**) and Endocrine portion (**Ep**).

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors are grateful to the Department of Biochemistry, Covenant University and Department of Biochemistry Olabisi Onabanjo University for providing laboratory facilities for this study.

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