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Pancreatic Histological Changes of Morizella® Juice

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Abstract

Background
Morizella juice is a nutritional supplement juice prepared from *Moringa oleifera* leaves and *Hibiscus sabdariffa* (HS) calyces. The juice is believed to have stabilizing effect on blood pressure (BP), anxiety, and to control glucose levels in some cases of diabetes. However, it is not recommended for consumption by infants and people with low blood pressure.

Broad objective
The aim of the current study was to evaluate the pancreatic histological changes in rats fed with Morizella juice.

Materials and Methods
A total of 18 healthy male and female Wistar rats, weighting between 183 and 224mg of 2-3 months old were used. The experiments were divided into short-term, mid-term and long-term test of six animals per group. Animals were sacrificed, the pancreatic tissues were harvested and histologically processed. Sections were examined and photographed using light microscope with in-built camera, and the number of Islet cells were measured in 40 high-power fields.

Results
Microscopic investigation of the pancreatic tissue in both treated groups revealed morphological changes in hormone-secreting endocrine tissues as evidenced by putative proliferation of Islets of Langerhans when compared with the control group. The number of Islet cells was significantly higher ($p < 0.05$) in all treated groups at the dosages of 175 and 350 mg/kg body weight. It is important to note that our results also showed the increase in number of intra-islets capillaries in all treated rats.

Conclusions
The beneficial effect of Morizella juice was observed in rats in all doses studied herein. It was found to increase the number of pancreatic endocrine cells as evidenced by pancreatic tissue proliferation with significant increase in intra-islet vascularization.

Recommendations
Further research aiming at the evaluation of histological changes of Morizella juice by using special stains for detection of the proliferation of islet cells and to recognize the β-cells of islets is recommended.

Keywords: *Morizella juice, Moringa oleifera, Proliferation, Hibiscus sabdariffa, Tanzania.*

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Introduction

Morizella Juice is believed to have stabilizing effect on blood pressure, anxiety, and to control glucose levels in some cases of diabetes (1). The juice is prepared from *Hibiscus sabdariffa* calyces and *Moringa oleifera* leaves in a four to two ratio (2).

Moringa is extensively promoted worldwide for a nutritional supplement since it is rich in protein, in minerals (iron and calcium) and in vitamins C and carotene (3). There are thirteen known Moringa species. However, the focus of this study was on *Moringa oleifera*. Moringa species were postulated to have a remarkable range of medicinal uses and high nutritional value (4). Moringa leaves have been shown to be beneficial in several chronic conditions, including hypercholesterolemia, high blood pressure, diabetes, insulin resistance, non-alcoholic liver disease, cancer and overall inflammation (5).

There are more than 300 species of Hibiscus, among numerous verities of them, *Hibiscus altissima* and *Hibiscus sabdariffa* are the commonest (6). Some species of Hibiscus possess certain medicinal properties of which *Hibiscus sabdariffa* is one (7). In vitro studies show that *Hibiscus sabdariffa* has antioxidant properties, however, these studies provide little guidance for animal and human studies through their lack of attention to cultivation, preparation and consumption patterns (8).

The hallmark feature of diabetes mellitus is the constant elevation of blood glucose level that occurs due to the destruction of pancreatic β-cell or the loss of cell responsiveness to insulin (9). Hyperglycemia can be managed initially with oral agents and insulin therapy. These synthetic agents, however, may produce some serious side effects and are also relatively expensive for developing countries (10). Therefore, searching for effective, low-cost hypoglycemic agents with fewer side effects is important.

Morizella juice is believed to have stabilizing effect on glucose levels in some cases of diabetes (1), however, till date there is no scientific documentation to support this hypothesis. Thus, the purpose of the current study was to document the effects of the juice to the pancreatic tissue.

Material and Methods

This investigation was set to be a true experimental study design that was conducted at Muhimbili University of Health and Allied Sciences (MUHAS) in Dar es salaam, Tanzania (TZ).

Experimental animals

A total of 18 healthy male and female rats of the Wistar strain weighing 183 - 224g with age range of 2-3 months were used. The animals were purchased from the animal breeding unit of the Institute of Traditional Medicine (ITM) at MUHAS, Tanzania. All animals were kept under standard conditions (at the room temperature 22°C ± 2°C), with natural 12 hours light/12 hours dark cycle. Animals were fed standard rat diet (Hill Pellet Broiler Feeds) and were allowed free access to water. The animals were acclimatized to laboratory conditions for one week prior to the experiment to alleviate any non-specific stress.

Preparation of administered extract

About 175 mg of the powdered Morizella extract was obtained by drying a 500 ml of Morizella juice using a freeze dryer. The extract was then transformed from human expected beneficial dose into animal equivalent dose (AED) according to Food and Drug Administration (FDA) draft guidelines (11). The AED can be calculated on the basis of body surface area by either dividing or multiplying the human dose (mg/kg) by the correction factor (Km) ratio. The Km ratio is the value obtained by dividing the animal Km factor by human Km factor or vice versa (12).

Experimental design and ethical considerations

A completely randomized block design (CRBD) was used for the experiment. The study was conducted in accordance with the ethical guidelines for investigations in laboratory animals and was approved (Ref. No. DA.25/11/01) by MUHAS ethical committee. Animals were divided into three main groups (A, B and C) according to the dose size. The extract was dissolved in distilled water and given to the animals orally via gavage. Group A and Group B received 175 and 350 mg/kg body weight, respectively. Group C received distilled water. The experiments were divided into short-term (14 days), mid-term (28 days) and long-term (90 days) test of six animals per group. In all tests, the pancreatic histological changes of Morizella juice in Wistar rats were evaluated (by the investigators with the assistance of the

expert from the histopathology unit, one of the units in the diagnostic laboratory department at Muhimbili National Hospital, Tanzania) at dosages of 175 and 350 mg/kg body weight orally by using oral gavage for a treated group and distilled water for a control group. The application of extract in short-term test occurred once. On the other hand, in the mid-term and long-term tests, the application occurred daily. The evaluation of short-term test was 14 days. Each group (A and B) underwent application of the extract once at day 1 and sacrificed at day 14 of the experimental period. For a mid-term test, each group (A and B) was applied with the extract once daily for 28 days, and sacrificed at day 28 of the experimental period. For a long-term test, each group (A and B) was applied with the extract once daily for 90 days, and sacrificed at day 90 of the experimental period.

Clinical and behavioral analysis

All animals were regularly and individually observed for any physical, food intake, behavioral alterations and signs of abnormalities after dosing for the first 24 hours, with special attention being given during the first four hours. Thereafter, the observation was continued daily for duration of 14, 28 and 90 days for the short-term, mid-term and long-term test, respectively.

Histological analysis

At the end of each experimental period, the six rats were euthanized as per the recommended guidelines. After manual evisceration, the visceral organs from the selected rats were picked and processed for histological analysis. Sections of the pancreas, were selected and processed for the light microscopy in order to observe any histological variation (normal or abnormal features) for treated and control groups. The excised organs were cleared of the adhering connective tissue, fixed for 24 hours by immersion in equal parts of 10% Neutral buffered formalin. Thereafter, the fixed tissue was dehydrated in ascending grades of ethanol, cleared in xylene and embedded in liquid paraffin wax. The tissues were sectioned at 5µm using the Heitz 150 rotary microtome (Cambridge model). The sections were then stained using Erlich's Haematoxylin and Eosin (H&E) staining technique using Baker and Silverton method (7). Sections were examined and photographed using a Leica® DM 750 swift light microscope with the in-built camera (Icc50 HD-47142065). To conduct morphometric analysis, scattered Islet cells were quantified in randomly selected areas of the pancreatic tissues in both treated and control groups in 40 high-power fields (HPF) using ImageJ Version 1.52k program.

Statistical analysis

The data obtained were statistically analyzed by using Statistical Package for Social Science (SPSS) software version 20. The values were expressed as mean ± standard deviation (SD) for different parameters. The effect of Morizella extract on the number of islets of Langerhans per area was analyzed by Student's unpaired *t*-test to compare the differences of the means between and within the groups. All variables were measured for significance using the range in 95% confidence interval and the p value from one-way analysis of variance (ANOVA) with confidence level set at ≤ 0.05% to test for significance between the mean values. Systematic error was calculated by paired measurement comparisons with a t-test.

Results

Short-term (14 days) test

General and behavioral observations

Following 14 days of Morizella extract administration at dosages of both 175 and 350 mg/kg body weight, the animals were observed in the first 24 hours, with special attention being given during the first four hours. Thereafter, observation continued daily for a total of 14 days. Both animals in treated and non-treated groups were normal and they did not show any significant changes in behavior, fur erection, breathing, impairment in food intake, water consumption, and postural abnormalities. In the treated groups, in the first six hours after applying the extract rapid heartbeat was observed but then normalized. This may be due to the stress of handling.

Histological examination

Microscopic investigation of the pancreatic tissue in both 175 and 350 mg/kg body weight treated groups revealed morphological changes in hormone-secreting endocrine tissues as evidenced by putative proliferation of Islets cells when compared with the control group. The Islets appeared lightly stained than the surrounding acinar cells (Figure 1). It is important to note that our results also showed the increase in number of intra-islets capillaries in all treated rats (**Figure 1**). It was found that the extract significantly increased the number of Islets of Langerhans in both treated groups, however, not significantly different in the group treated with 175 mg/kg body weight (**Table 1**).

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Table 1: Effect of Morizella juice on pancreatic tissues in 14 days

Groups	n_1	n_2	Mean number of Islets/40 HPF	p-value	Average area of islets (mm^2)/40 HPF
A	14	17	15.5 ± 2.12	**0.403**	0.0027 ± 0.0004
B	26	23	24.5 ± 2.12**	**0.017**	0.0025 ± 0.0002
C	14	16	15.0 ± 1.41		0.0028 ± 0.0002

***Significant against Group C, n1: first animal; n2: second animal, (A) treated with 175 mg/kg b.w, (B) treated with 350 mg/kg b.w, (C) control group, HPF: High-power field. All results are expressed as means ± S.D at p<0.05.*

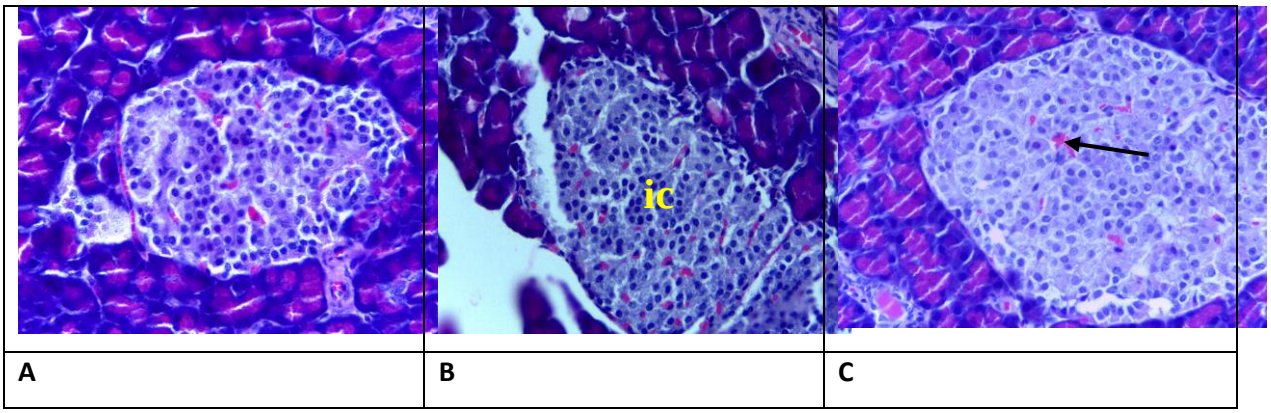

Figure 1: (A) Section of a pancreas of healthy rat treated with 175 and 350 mg/kg b.w of morizella extract showing partially increment of islets cells (ic) and intra-islets capillaries (arrow), (B) Section of a pancreas of healthy rat treated with 350 mg/kg b.w of morizella extract showing an obvious increment of islets cells (ic) and intra-islets capillaries (arrow), and (C) Pancreatic section of a control rat showing no histological changes of islets cells (IC) and intra-islets capillaries (arrow) (H&E ×40).

Mid-term test (28 days)

General and behavioral observations

Following 28 days of Morizella extract administration at dosages of both 175 and 350 mg/kg body weight, the behavioral patterns were observed in the first 24 hours, with special attention being given during the first four hours. Thereafter, observation was continued daily for a total of 28 days. The animals in both treated and non-treated groups were normal and did not display any significant changes in behavior, fur erection, breathing, impairment in food intake and water consumption, and postural abnormalities.

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Histological examination

Both groups treated with 175 and 350 mg/kg body weight revealed histological changes in endocrine cells and intra-islet vasculatures when were compared to control group (Figure 2). Microscopic investigation of pancreatic section of the control group showed the normal appearance with preserved pancreatic islet architecture. The average number of Islet cells per selected areas of the pancreatic tissues in both 175 and 350 mg/kg treated groups was significantly different (p<0.005) when compared to the control group (Table 2).

Table 2: Effect of Morizella juice on pancreatic tissues in 28 days.

Treatment Group	n_1	n_2	Mean number of Islets/40 HPF	p-value	Average area of Islets (mm^2)/40 HPF
A	23	19	21.0 ± 2.83**	**0.028**	0.0020 ± 0.0003
B	20	21	20.5 ± 0.71**	**0.008**	0.0029 ± 0.0001
C	11	13	12.0 ± 1.41		0.0021 ± 0.0002

***Significant against Group C, n1: first animal; n2: second animal, (A) treated with 175 mg/kg b.w, (B) treated with 350 mg/kg b.w, (C) control group, HPF: High-power field. All results are expressed as means ± S.D at p<0.05.*

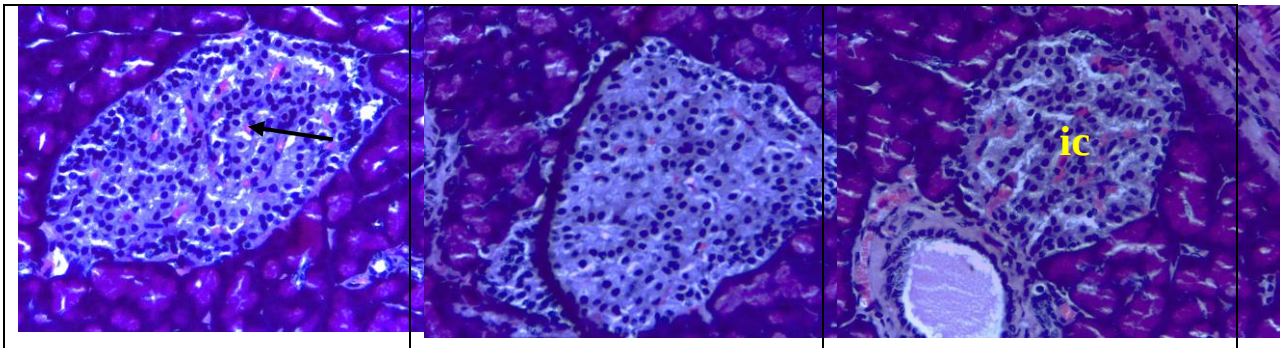

Figure 2: (A) Section of a pancreas of healthy rat treated with 175 and 350 mg/kg b.w of morizella extract showing partially increment of islets cells (ic) and intra-islets capillaries (arrow), (B) Section of a pancreas of healthy rat treated with 350 mg/kg b.w of morizella extract showing an obvious increment of islets cells (ic) and intra-islets capillaries (arrow), and (C) Pancreatic section of a control rat showing no histological changes of islets cells (ic) and intra-islets capillaries (arrow) (H&E ×40).

Long-term (90 days) test

General and behavioral observations

Following 90 days of Morizella extract administration at dosages of both 175 and 350 mg/kg body weight for group A and B, respectively, the behavioral patterns were observed in the first 24 hours, with special attention being given during the first four hours. Thereafter, observation was continued daily for a total of 90 days. The animals in both treated and non-treated groups were normal and did not display any significant clinical signs in behavior, fur erection, breathing, impairment in food intake and water consumption, and postural abnormalities.

Histological examination

The animals in both treated groups showed histological changes of endocrine Islets cells and intra-islet vasculature when they were compared with a control group. The islets appeared lightly stained than the surrounding acinar cells (Figure 3). Concerning microscopic histological examination, Morizella extract significantly increased the number of islets of Langerhans in both treated groups. However, a significant different (p<0.05) was observed only in the group treated with 350 mg/kg body weight (Table 3).

Table 3: Effect of Morizella juice on pancreatic tissues in 90 days.

Treatment Group	n_1	n_2	Mean number of Islets/40 HPF	p-value	Average area of Islets (mm^2)/40 HPF
A	21	17	19.0 ± 2.82	**0.304**	0.0019 ± 0.0003
B	35	33	34.0 ± 1.41**	**0.005**	0.0046 ± 0.0002
C	16	19	17.5 ± 2.12		0.0027 ± 0.0004

*** Significant against Group C, n1: first animal; n2: second animal, (A) treated with 175 mg/kg b.w, (B) treated with 350 mg/kg b.w, (C) control group, HPF: High-power field. All results are expressed as means ± S.D at p<0.05.*

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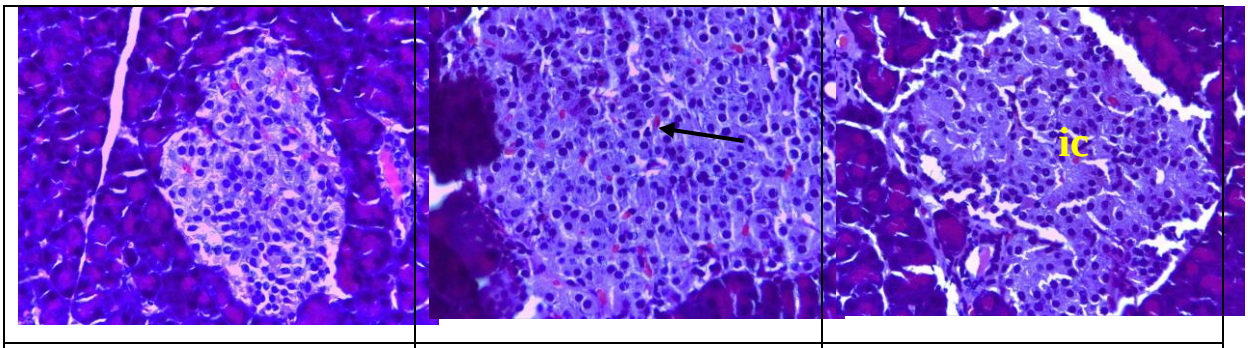

A	B	C

Figure 3: (A) Section of a pancreas of healthy rat treated with 175 and 350 mg/kg b.w of morizella extract showing partially increment of islets cells (ic) and intra-islets capillaries (arrow), (B) Section of a pancreas of healthy rat treated with 350 mg/kg b.w of morizella extract showing an obvious increment of islets cells (ic) and intra-islets capillaries (arrow), and (C) Pancreatic section of a control rat showing no histological changes of islets cells (ic) and intra-islets capillaries (arrow) (H&E ×40).

Discussion

Regardless of the pharmacological benefits of the Morizella extract, detailed knowledge about histological evaluation of this medicinal juice is lacking. The histological examinations provide information to strengthen the findings on biochemical and hematological parameters (13). Hence, the current study was undertaken to evaluate and focus on the histological changes in an animal model fed with Morizella extract. Apparently, the histological examination of the pancreas of treated rats revealed morphological changes of the hormone-secreting endocrine cells, which showed the marked putative proliferation of pancreatic Islets of Langerhans in all oral tests. It is important to note that we also found an increase in intra-islets capillaries in all groups treated with both 175 and 350 mg/kg of Morizella extract. Our results clearly suggested that Morizella extract could be implicated in the production and development of human islets cell population and this may lead to development of a novel strategy for diabetes treatments.

Current results were difficult to compare directly with other *Moringa oleifera* and *Hibiscus sabdariffa* extract studies as ours was the first to evaluate the extract from a combination of the different ratio of these two plants. However, the study results agreed with those from earlier studies around the world which found out that aqueous leaf extracts of *Moringa*

Oleifera (14) and components extracted from Roselle extract caused a decrease in blood glucose and increase in the number of β-cells (15). Additionally, the findings from this study are in agreement with those of other previous studies, which reported the plant leaves being relatively safe for both nutritional and medicine uses (16).

The general mechanism by which Morizella extract increased the number of islet cells is not clear but it was probably the result of replication of existing mature β-cells and differentiation (or neogenesis) by ductal or intra-islet pancreatic precursor cells. Unfortunately, it was difficult in our experiment to differentiate β-cells from α-cells on Hematoxylin and Eosin staining tissue. Nevertheless, our results showed the increase in Islets cells that were related to the beneficial effect of Morizella juice in animal model. This is the first study to document the histological changes in rats fed with Morizella extract at the dose of 175 and 350 mg/kg body weight. Further research aimed at the evaluation of histological changes of Morizella juice by using special stains for detection of the proliferation of islet cells and to recognize the β-cells of islets is recommended.

Conclusion

Accordingly, in the light of these findings, we may conclude that the beneficial effects of Morizella extract was observed in all doses studied herein and did not produce any evident symptoms in any of the oral test studies. Furthermore, the data were obtained in order to increase the confidence in its safety to humans for the use in the development of pharmaceuticals.

Limitation

This was a study with a short duration such that chronic toxicity study could not be carried at.

Competing Interest

The authors have no conflicting financial interest.

Authors' Contributions

AKH performed the experiments, analyzed the data and wrote the manuscript. ADR contributed in designing the study, reviewed the data and manuscript. All authors discussed the results and approved the manuscript.

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List of Abbreviations

AED	Animal Equivalent Dose
BP	Blood Pressure
CRBD	Completely Randomized Block Design
FDA	Food and Drug Administration
HPF	High-Power Field
HS	Hibiscus sabdariffa
ITM	Instituted of Traditional Medicine
MUHAS	Muhimbili University of Health and Allied Sciences
S.D	Standard Deviation.
TFDA	Tanzania Food and Drugs Authority
TZ	Tanzania

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