

## Assessment of Pancreatic and Hepatic Histological Changes in Streptozotocin-Induced Diabetic Rats Fed with Morizella Juice

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### ABSTRACT

**Background:** *Hibiscus sabdariffa* and *Moringa oleifera* has been reported to have ant diabetic effect on hyperglycemic animals. However, there are no reports on its histological and physiological changes in diabetic animals.

**Objective:** To assess the pancreatic and hepatic histological changes in streptozotocin-induced diabetic rats fed with Morizella juice.

**Methods:** A Completely Randomized Block Design (CRBD) was used for this study, 40 adult male Wistar rats, 9 to 10 weeks with a bodyweight 190g – 250g were selected for the study. The animals were divided into four main groups (A, B, C, and D), each group was further subdivided into two groups with 5 rats. The experiments were divided into acute and sub-acute tests of 10 animals per group. At the end of the experiment, animals were sacrificed, pancreatic and liver tissues were harvested and histologically processed. Sections were examined and photographed using a light microscope (Leica® DM 750) with an in-built camera ((Icc50 HD-47142065) and the number of Islet cells was measured in 40 high-power fields.

**Results:** Microscopic structures of the pancreas revealed that the number of Islets of Langerhans was significantly higher in the Morizella treated groups at the dosages of 50mg/kg and 100 mg/kg body weight. The microscopic structure of the liver in both treated groups presented with normal histological architecture when compared with both control groups

**Conclusion:** There was a dose-dependent increase of islets of Langerhans cells in the acute and sub-acute group treated with Morizella extracts compared to the control group. Additionally, the liver architecture was reversed back to the normal in Morizella-treated group.

**Keywords:** *Hibiscus sabdariffa*, *Moringa oleifera*, Streptozotocin, Diabetic rats

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### Introduction

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces (WHO, 2016). The complication of diabetics included retinopathy, neuropathy, nephropathy, accelerated atherosclerosis, and other cardiovascular diseases, and that is due to persistent hyperglycemia and alterations in protein and lipids metabolism (Nyondo *et al.*, 2020). There were 108 million people with diabetes in 1980 but in 2014 people with diabetes were estimated to be 422 million which is a 390.74 % increase. Forty percent of people with diabetes are expected to have premature death before the age of 70 (WHO, 2016).

Africa has more communicable diseases compare to noncommunicable diseases, but it has been estimated that by the year 2020 the non-communicable diseases will strip communicable diseases as a cause of death (Feachem and Jamison, 1991). There is a significant increase in the prevalence of diabetes in rural areas as well as urban areas (Animaw and Seyoum, 2017).

Morizella<sup>®</sup> juice contains two contents; *Moringa oleifera* and *Hibiscus sabdariffa*; these two plants are indigenous, edible, medicinal plants used in Tanzania for the treatments of several diseases including diabetes. The mixture affects the liver, cardiovascular system, body homeostasis such as regulating body insulin and also works better as nutritional supplements to pregnant mothers, undernourished children as well as those with immune-compromised diseases. Despite those major advantages but it is not recommended for people with high blood pressure (F. Mwimanzi, 2008). *Moringa* tree belongs to the family *Moringaceae* which are in a genus of *Moringa* at beginning of the 20<sup>th</sup> Century this tree was introduced to Eastern African Countries from India (Foild, Makkar, and Becker, 2001). *M. oleifera* has been used since ancient time and given a name of miracle tree, from several plants which were tested for anti-diabetic effect *M. oleifera* shows a promising effect (Khan et al, 2017).

Rosella has been given several names depending on the country (area) those names including roeselle, razelle, red sorrel and lalambari, it is an annual herbaceous shrub belonging to the family *Malvaceae*, it is believed to be originated in Sudan 4000BC and carried out to the other countries including Europeans countries. It is an annual crop that can grow in tropical and subtropical climate (Mohamed et al, 2012). *H. sabdariffa* shows to have more effect in reducing blood glucose compared to the metformin as inhibition of enzyme phosphoenolpyruvate carboxykinase (PEPCK) have a low score and better inhibition potential (Nerdy, 2015).

This study aimed to assess the liver and pancreas histological changes and exploring scientific facts on the effect of the juice even though the mixture has undergone many studies around the world. The majority of studies conducted have used only one content of the Morizella<sup>®</sup> juice, (*H. sabdariffa* or *M. oleifera*). Also, this study was geared to find out the effect of Morizella<sup>®</sup> juice on the liver and pancreas so that it can be modernized and used as a modern drug and also to be an eye-opener to other scientists in the future studies of this nature. Studies conducted in several parts of the world concerning the effect of Morizella<sup>®</sup> juice were done by using one content of the mixture so this study also gave a comparative analysis with other studies.

## **Methodology**

### **Materials and reagents**

Streptozotocin (Sigma-Aldrich, Germany), Zinc-insulin suspension (Lente insulin) [Monotard, Novo Nordisk, Bagsvaerd, Denmark], Glucometer (Medisign<sup>®</sup>, Tianjin, Empecs Medical Device Co., Ltd., Tianjin, China), pH meter, and carboxymethyl cellulose (CMC) were used. Reagents and materials were sourced commercially.

### **Plant material**

The extract was prepared by blending *M. oleifera* and *H. sabdariffa* calyces by ITM. Using a ratio of 2:1 of *M. Oleifera* and *H. Sabdariffa* the extract was prepared and 10g of powder was obtained. Morizella powder was kept in an airtight plastic container and stored at 5 °C until used. Morizella powder was suspended in warm distilled water (100 mg/1 ml and 50 mg/1 ml) and was given orally by using oral gavages at a dose of 50 mg/kg and 100 mg/kg.

### **Study Design and area**

A completely randomized block design (CRBD) was used for the experiment, the adult healthy male Wistar rats of 9 to 12 weeks with a bodyweight (220g ± 30g) were selected for the study. Rats were maintained under the standard environmental conditions of a temperature of 22°C (± 3°C) and relative humidity of at least 30% and did not exceed 70%. Artificial light was used and a cycle of 12 hours of light and 12 hours of dark cycle was followed. The animals were fed with conventional food from the

laboratory with unlimited water supply. One week before dosing acclimatization of rats was done. The rats received humane care, according to the Muhimbili Institute of Traditional Medicine guide for the care and use of laboratory animals.

### **Animal dosage of Morizella Extract**

The dose of 50mg/kg and 100mg/kg body weight has been used repeatedly in different kinds of literature. From Previous studies initially, an oral dose of 50mg/kg and 100mg/kg of Morizella was selected which was administered for 14 and 28 days for assessment of histological changes of the pancreas and liver of STZ induced diabetic rats.

As 50mg/kg and 100mg/kg showed better activity, so those doses were used subsequently to evaluate the protective effect of *H. Subdariffa* and *M. oleifara* in STZ induced diabetic rats, A dose of 50mg/kg and 100mg/kg b.w of the tested drug was selected (Md Idris *et al.*, 2012; Al-Malki and El Rabey, 2015). So this study also adopted those two doses of 50mg/kg and 100mg/kg. Morizella extract of 50mg/kg b.w and 100mg/kg b.w sugar-free was considered as a normal and higher human dose per day respectively.

### **Study population, sample size, and selection**

The sample size was obtained from the previous studies which involved the same animal species, strain, age, gender, and health status (Festing, 2018). The sample size of 40 male Wistar rats was selected from two previous studies (Nafizah *et al.*, 2017; Albrahim and Binobead, 2018). The study area was at the School of Medicine (MUHAS).

About 40 male Wistar rats were an optimal number used in this study. The animal involved was more than the sample size to have enough numbers at the end of the study for data analysis. Rats were obtained from the animal breeding unit of ITM) at Muhimbili University of Health and Allied Sciences (MUHAS). During this study, the American Veterinary Medical Association guidelines for the depopulation of animals were followed (Leary *et al.*, 2019).

### **Induction of insulin-dependent diabetes mellitus (IDDM)**

A single intraperitoneal (i.p) injection of 65mg/kg b.w Streptozotocin (STZ) dissolved in 0.1 citrate buffer (pH 6.3) was used to induce type 1 diabetes mellitus to Wistar rats. The animals were allowed to take 10% sucrose water immediately after they have been induced diabetes with streptozotocin for 24 hours to avoid death by acute hypoglycemia. The rats were induced by diabetes intraperitoneal after 12 hours of fasting. Diabetes was confirmed by measuring the blood glucose concentration 72 h after injection. The blood sample was taken from the rats' tail vein. The rats with blood glucose above >16 mol/L were considered to be diabetic and selected for the experiment (Nazratun Nafizah *et al.*, 2017).

### **Experimental design**

Animals were divided into 4 groups; Group A and B which were experimental groups, Group C which was Morizella group, and Group D which was the Control group. Each group (A, B, C, and D) was further subdivided into A1, A2, B1, B2, C1, C2, D1, and D2 subgroups, Group A1, B1, and C1 received 50mg/kg of Morizella extract. Group A2, B2, and C2 received 100mg/kg of Morizella extract. Subgroup D1 (Positive control group) received insulin 1IU/kg and subgroup D2 (Negative control group) received distilled water. Each group included 10 male Wistar rats with 5 rats in each subgroup. The experiment was categorized into the acute study and sub-acute study.

### **Acute study**

This test involved Streptozotocin induced diabetic rats, animals were given a single dose of the Morizella extract of 50mg/kg b.w and 100mg/kg b.w. After dosing animals were kept under close monitoring. Group A1 was given 50mg/kg b.w of Morizella extract. The dose was given to animals on the first day only. Group A2 was given 100mg/kg, it was given on the first day only. After administration of the dose very special observation of behavioral changes as well as toxic signs and more emphasis was on the first 4 hours after administering of dose. The observation went on for all 14 days of the experiment. All experimental animals were observed closely for any acute toxicity responses.

### **Sub-acute study**

This test involved groups B, C, and D whereby groups B and D were STZ induced diabetic groups while group C was a non-induced diabetic group. Each group involved 10 male Wistar rats.

Group B rats were randomly grouped into an experimental of 50mg/kg (normal dose) and an experimental of 100mg/kg (high dose) of Morizella extract. The animals in this experimental group were treated with Morizella extract with the intervals of 24 hours for 28 days. Onset, duration, and severity of toxic signs were factored for determination between the time interval and dosing of each animal at each level. All animals had free access to determine standard pellets and tap water. All treatments were administered via oral gavages. All groups were closely observed for any physical, food intake, water intake, behavioral alterations, and signs of abnormalities throughout the study. Treatment of animals in the next dose was given after the previously dosed animals survived.

Group C included non-induced diabetic rats which given 50mg/kg b.w and 100mg/kg b.w of Morizella extract for C1 and C2, respectively for 28 days. Group D involved STZ induced diabetic rats which were given 1IU/kg of insulin and distilled water for D1 and D2, respectively for 28 days.

### **Sample Collections and Histopathological Analysis**

At the end of the experimental period, 24 rats (6 in each group) were selected and chloroform as the euthanized gas agent was used as per AVMA guidelines for the depopulation of animals: 2019 edition. After manual evisceration, visceral organs (liver and pancreas) from the selected rats were picked and processed for histopathological analysis.

### **Data management**

All quantitative data were packed and analyzed by SPSS (version 20) statistical software. All values of parameters (number density of cell nuclei and size) were expressed in mean  $\pm$  SD (standard deviation). Treatment over time was compared between control and treated groups by using a one-way analysis of variance (ANOVA), followed by Turkey post hoc test to determine their level of significance. Differences at  $p < 0.05$  were considered statistically significant. The parameters collected (normal and abnormal features), the number and size of pancreatic and hepatic cells were recorded.

### **Ethical clearance**

Ethical clearance to conduct the study and also use laboratory animals was obtained from the MUHAS ethical committee.

## **Results**

### **Food and water intake**

The average amount of water taken by diabetic rats supplemented with Morizella extract was low compared with the negative control group. The amount of food was higher in diabetic rats received



distilled water ( $p < 0.05$ ) compared to the rats which were supplemented with insulin and Morizella extract. (Table 1).

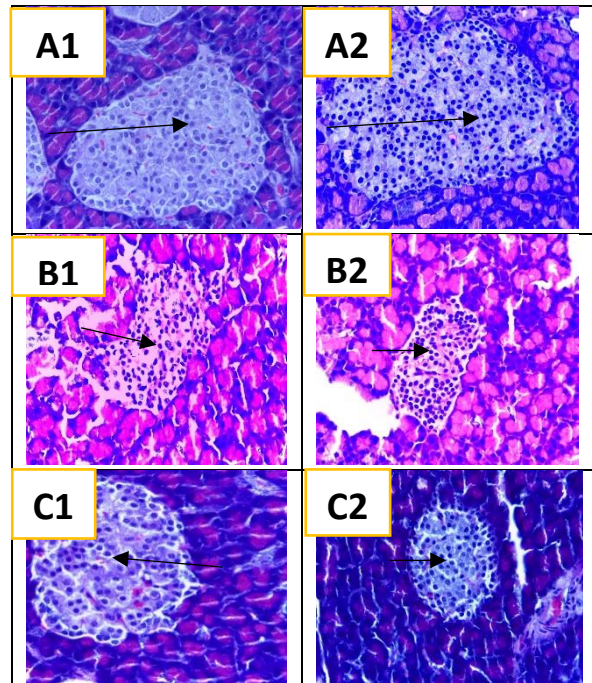
GROUP	SUBGROUP	FOOD (g)	WATER (ml)	N	P – VALUE
Acute (A)	A1	44.64 ± 11.24*	1.28 ± 15.89*	5	0.00
	A2	38.35 ± 10.39*	55.64 ± 14.64*	5	0.00
SUB-ACUTE (B)	B1	33.96 ± 6.69*	47.53 ± 7.68*	5	0.00
	B2	32.30 ± 4.6 *	45.93 ± 5.82*	5	0.00
MORIZELLA (C)	C1	31.03 ± 0.66*	44.23 ± 1.03*	5	0.00
	C2	31.28 ± 0.71 *	44.53 ± 1.57*	5	0.00
CONTROL (D)	D1	30.07 ± 5.37*	44.60 ± 0.91*	5	0.00
	D2	57.53 ± 1.99	77.71 ± 2.41	5	0.00

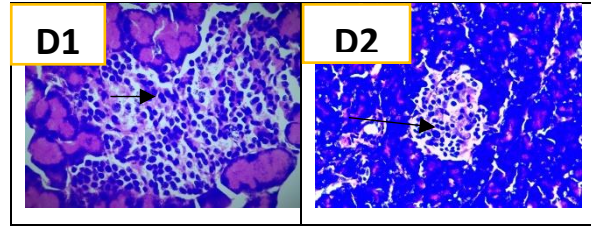
A1 represents the subgroup administered with 50mg/kg and A2 represents the subgroup administered 100mg/kg of Morizella extract for 14 days. B1 represents the subgroup administered with 50mg/kg and B2 represents the subgroup administered 100mg/kg of Morizella extract for 28 days. C1 represents the subgroup administered with 50mg/kg and C2 represents the subgroup administered 100mg/kg of Morizella extract for 28 days. D1 represents the subgroup administered insulin for 28 days and D2 represents the subgroup received distilled water for 28 days. (Values are presented as Mean ± SD and \*represents statistical significance versus negative control ( $p \leq 0.05$ )).

### Histological examination

As illustrated in figure 1, the pancreas of diabetic rats fed with Morizella extract 50mg/kg and 100mg/kg showed normal islets with a regular elliptical shape and clear boundary. Table 2, shows the increased number of pancreatic islets cells following the administration of Morizella extract.

As illustrated in figure 2, the liver of the diabetic negative control group showed spotty necrosis, poor arrangement of hepatic cells, and irregular nuclei with some swelling.



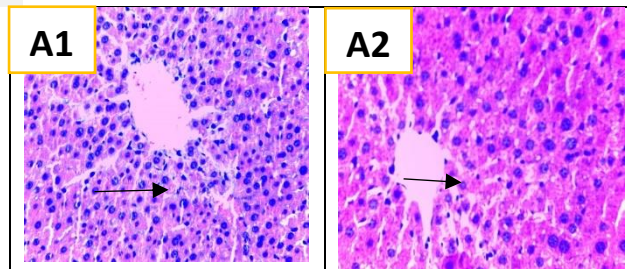


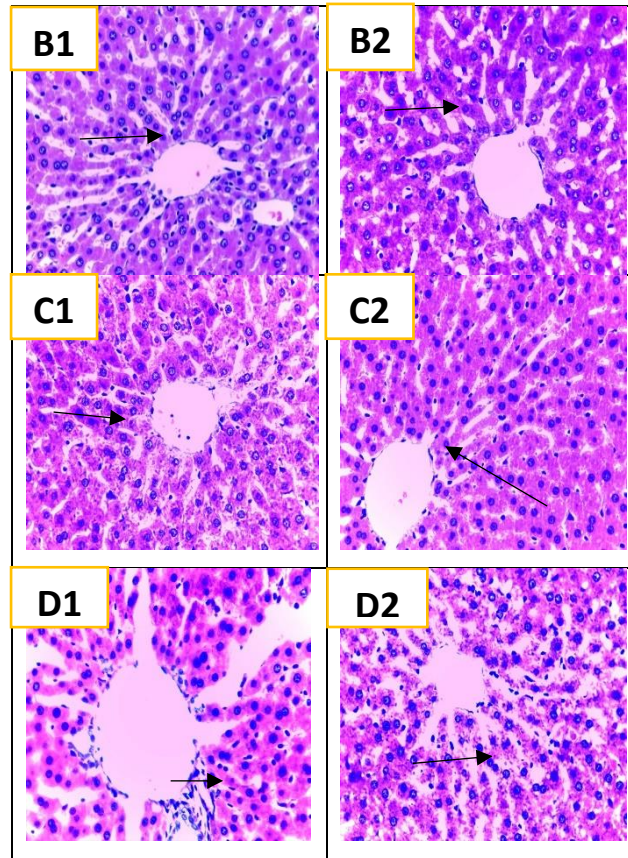
**Figure 1: Pancreas Histopathology**

**(A1 & A2)** STZ induced diabetic rats fed with 50mg/kg and 100mg/kg b.w of Morizella extract once showing perfect islets with a regular elliptical shape and clear boundary between it and surrounding acinar cells (H & E 40X). **(B1 & B2)** STZ induced diabetic rats pancreas fed with 50mg/kg and 100mg/kg b.w of Morizella extract daily for 28 days showing normal islets of Langerhans with some degeneration of  $\beta$  cells in the center were noticed (H & E 40X). **(C1 & C2)** Morizella group fed with 50mg/kg and 100mg/kg showing normal islets of Langerhans with pale rounded and ovoid  $\beta$  cells in the center, embedded in an exocrine portion of the pancreas (H & E 40x). **(D1)** STZ induced diabetic rats were treated with insulin showing lost ellipse of the islets with unclear boundary with the surrounding acinar (H & E 40x). **(D2)** STZ induced diabetic rats received distilled water showing shrunk (degenerated) round islet with an irregular boundary, lymphocytes, and unclear acinar cells' details (H & E 40x).

**Table 2: Effect of Morizella extract on histological changes of islets of Langerhans in the pancreas**

Groups	Subgroup and dosage	Number of Islets/40 HPF	Average area of Islets (mm <sup>2</sup> )/40 HPF
<b>A (Acute group)</b>	A1 (50mg/kg)	7.80 ± 0.83	0.0027 ± 0.0004
	A2 (100mg/kg)	10.60 ± 0.89	0.0018 ± 0.0002
<b>B (Sub Acute group)</b>	B1 (50mg/kg)	12.80 ± 1.64	0.0027 ± 0.0004
	B2 (100mg/kg)	22.80 ± 0.83	0.0018 ± 0.0002
<b>C (Morizella Group)</b>	C1 (50mg/kg)	14.20 ± 0.83	0.0046 ± 0.0002
	C2 (100mg/kg)	24.00 ± 1.00	0.0047 ± 0.0003
<b>D (Control Group)</b>	D1 (Insulin 1IU/kg)	14.20 ± 0.83	0.0034 ± 0.0003
	D2 (Distilled water)	3.20 ± 1.30	0.0032 ± 0.0003





**Figure 2: Liver Histopathology**

**(A1 & A2)** STZ induced diabetic rats fed with 50mg/kg and 100mg/kg of Morizella extract showed the normal arrangement of hepatic cells (rope-like distribution) about the central vein, without obvious hepatic sinusoids. Individual cells were of varying sizes and there were some swollen of cells (H & E 40x). **(B1 & B2)** STZ induced diabetic rats fed with 50mg/kg and 100mg/kg of Morizella extract showing a perfect arrangement of hepatic cells with obvious hepatic sinusoids, fairly uniform cells, and nuclear sizes with distinct nuclear membrane (Black arrow) (H & E 40x). **(C1 & C2)**.

The Morizella group rats fed with 50mg/kg and 100mg/kg of Morizella extract showing normal liver architecture. (Black arrow) (H & E 40x). **(D1)** STZ induced diabetic rats (Positive control group) were given insulin injection showing a fairly maintained arrangement of hepatic cells but massively dilated hepatic sinusoids. (Black arrow) (H & E 40x). **(D2)** STZ induced diabetic rats (Negative control group) received distilled water showing spotty necrosis, poor arrangement of hepatic cells, and irregular nuclei with some swelling. There was glycogen infiltration which caused vacuolation (Black arrow) (H & E 40x)

### Discussion

This study indicated that Morizella extracts turned the pathological liver and pancreas of STZ induced diabetic rats to normal architecture. There were dose-dependent benefits in rats used 50mg/kg and 100mg/kg, rats given 100mg/kg had more increase in pancreatic islets cells, and perfect liver architecture. The study conducted to evaluate the effect of *Punica gratum* peels powder on the STZ induced diabetic rats showed that the rats which supplemented with PGPP had progressive decrease water intake especially from day 5.

Food intake was decreased in the treatment group compared to the control group, the decrease was statistically significant (Saad et al., 2015). In the present study, the treatment groups



which received 50mg/kg and 100mg/kg of Morizella extract showed persistent decreases in water and food intake, especially from day 3 of treatment. The negative control group has a progressive increase in food and water intake after induction of diabetes to the end of the experimental period. The positive control group which received insulin showed a progressive decrease in food and water intake from day 5 to the end of the experimental period.

The *H. Sabdariffa* shows the remarkable decrease in blood glucose in Diabetic induced rats and also in histological examination showed increased activity of the pancreatic islets of Langerhans. Excessive free radicle can be cleared by flavonoid which is found in *H. sabdariffa*, which also can stimulate regeneration of Pancreatic  $\beta$  cells to secret insulin to lower blood glucose levels (Sunaryo, Hikmawanti, and Listyaningrum, 2019).

In the present study, Morizella extract which one of the constituents is *H. Sabdariffa* showed the histological changes of pancreatic cells in STZ induced diabetic rats, the groups which were STZ induced diabetic rats treated with Morizella extract 50mg/kg and 100mg/kg daily for 28 days show highly increase an average number of Islets cells compared to STZ induced diabetic rats received 50mg/kg and 100mg/kg single dose. Also, the groups treated with different doses of Morizella extract showed regeneration of pancreatic  $\beta$  cells compared with groups treated with insulin and group given distilled water which shows no regeneration. The Morizella group showed a significantly higher average number of Islets cells compared to other groups and this gives the evidence that *H. Sabdariffa* can cause histological changes.

The study of the Effect of *H. sabdariffa* calyx extract on carbon tetrachloride-induced liver damage showed that *H. sabdariffa* enhances recovery of liver damage by the restoration of ALT and AST (Dahiru and Aduwamai, 2016). *M. oleifera* has a protective effect on the pancreas structure which is affected by nicotine use and prolonged use of it can lead to a more beneficial effect (Omotoso et al., 2018).

In this study moreover, a microscopic investigation of the liver of the STZ induced diabetic rats treated with different doses of Morizella extract showed the ability to restore the liver morphological structures. The Liver of STZ induced diabetic rats treated with Morizella extract 50mg/kg and 100mg/kg showed normal liver architecture compared to the group which received insulin which showed a fair arrangement of hepatic cord with massively dilated sinusoids and the group which received distilled water which showed spotty necrosis, poor arrangement of hepatic cells. The Morizella group which received 50mg/kg and 100mg/kg showed normal liver architecture. The hepatocytes were normally arranged in all experimental groups which were treated with 50mg/100g and 100mg/100g of Morizella extract.

There are beneficial effects of the Morizella extract in STZ induced diabetic rats, it increases the average number of islets cells and restoration of the normal liver architecture as it was observed. Findings showed that the rats which received a high dose had more positive results than those who received the low dose. However, the induction of diabetes in rats does not mimic 100% the occurrence of diabetes in humans so we do suggest that other research should be conducted using a large number of animals and if possible humans for better results.

The price of the hypoglycemic drug is very high so 26% of patients in Tanzania government hospitals are unable to afford medicine and 10% in Tanzania private hospitals are unable to afford medicine, so great efforts are being made to find natural medicines that are cheaper and less harmful (Justin-Temu et al., 2009). So, this study gives room to develop the traditional drug which is safer and less costly.

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