

INSULIN PROFILING AND HISTO-PANCREATIC ARCHITECTURE IN STZ-INDUCED DIABETES MELLITUS RATS' MODEL TREATED WITH MISTLETOE AND DRUM-STICK AQUEOUS LEAF EXTRACTS

¹Adeeyo Olusola Atilade, ^{2*}Dare Babatunde Joseph, ³Yusuf Uthman Ademola, ⁴Okeniran Olatayo Segun, ⁵Ebiwonjumi Adetunji Segun and ⁶Ojo Waliu Adetunji

^{1,5,6}Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology.

²Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University.

³Department of Anatomy, School of Medicine and Health Sciences, Central Hospital Livingstone Mulungushi University, Zambia.

⁴Department of Anatomy, University of Gitwe, Rwanda.

*Corresponding Author: Dare Babatunde Joseph

Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University.

Article Received on 04/12/2017

Article Revised on 24/12/2017

Article Accepted on 14/01/2018

ABSTRACT

Reports showed that antioxidants protect against disabling effects of Diabetes mellitus by mopping up free oxygen and superoxide radicals. The study is therefore aimed at investigating some ameliorative effects of Mistletoe and Drum-stick leaf extracts on STZ-induced Diabetes mellitus on the pancreatic histo-architecture and insulin profile. Fifty four (54) Wistar rats of both sexes were selected randomly into six (6) groups (G₁-G₆) of nine (9) animals each. The animals were acclimatized for period of two (2) weeks. Hyperglycemia was induced in twenty seven (27) overnight-fasted randomly selected rats by a single intraperitoneal administration of Streptozotocin at 70 mg/kg bw. G₁ treated with 0.1 ml of phosphate buffer saline, G₂ diabetic treated with Mistletoe leaf extracts only, G₃ diabetic treated with Moringa leaf extracts only, G₄ diabetic without treatment, G₅ normoglycemic treated with Mistletoe leaf extracts only, G₆ normoglycemic treated with Moringa leaf extracts only. Administration was done for six (6) weeks, animals were sacrificed by euthanasia and pancreas was harvested, fixed in 10% formol saline for H/E, Masson's Trichrome and Von Giessen stains. A portion of pancreas was homogenized in phosphate buffer solution for estimation of pancreatic insulin concentrations using Mercodia ultra-sensitive rat insulin Elisa kit (Mercodia, Uppsala, Sweden). Mistletoe (105.22± 4.22 mg/dl) and Drum-stick (94.33± 5.62 mg/dl) significantly lowered blood glucose levels, increase in body weight and relative pancreatic weight were observed relative to the untreated diabetic rats. The Pancreatic Insulin levels of Mistletoe (3.4±0.186 µg/L) and Drum-stick (3.5±0.088 µg/L) treated hyperglycemic groups were not significantly different from normal control (3.8±0.25 µg/L) P>0.05. The pancreatic histo-architecture was maintained in the pancreatic islet of Drum-stick treated group with improved vascularization, increase in β cell mass. Elastic fibers were extensively deposited in the acini of untreated diabetic group. Thus aqueous leaf extracts of Mistletoe and Moringa maintained pancreatic islet morphology and insulin profiling.

KEY WORDS: hyperglycemia, Anti-diabetics, Antioxidants, Leaf Extracts, Pancreas and Wistar rats

BACKGROUND

Accumulating evidences suggest that cellular injury caused by free radicals contributes to the development of Diabetes mellitus (Exposito *et. al.*, 2002). Free radicals are either generated by cellular metabolism such as glycolysis, mitochondrial respiration and xenobiotic detoxication or by exogenous factors such as redox reaction. Some are extremely reactive and therefore interact with vital macromolecules of life including lipids, nucleic acids and proteins (Guzik, Mussa and Gastaldi, 2003). The cells have numerous defense systems (enzymatic and non-enzymatic antioxidants) to counteract the deleterious effects of reactive oxygen

the antioxidant enzymes (Gernet, Kale and Baquer. 2002).

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by glucose intolerance and fasting hyperglycemia (Kantarov *et. al.*, 2006). Diabetes mellitus is associated with disabling and life threatening complications such as retinopathy, nephropathy, hepatopathy and coronary artery diseases (Libman *et. al.*, 1993). Chronic hyperglycemic oxidative stress is implicated in the pathogenesis of these complications (Jeanette *et. al.*, 2005).

Previous reports showed that antioxidants protect from the disabling effects of Diabetes mellitus by mopping up free oxygen and superoxide radicals (Jeanette *et al.*, 2005). It has also been reported that depletion of antioxidant appears to be a major risk factor for developing complications in Diabetes mellitus, and that antioxidant supplements lowered the risk (Jeanette *et al.*, 2005). Besides, impaired insulin levels or action in Diabetes mellitus predisposes to dyslipidemia and increased risk of atherosclerosis (Rossetti and Goldberg, 2002).

Type I Diabetes is immune mediated and accounts for about 10% of all diabetic cases, affecting approximately 20 million people worldwide (Libman *et al.*, 1993). Type 2 insulin-resistant Diabetes mellitus accounts for about 90% of all Diabetics. It afflicts an estimated 6% of adult population, and its worldwide prevalence is expected to grow by 6% per annum (Amos *et al.*, 1997). Fruit juice of *Momordica charantia* reverses hyperglycemia in rats by decreasing gluconeogenesis and increasing insulin secretion (Shibib *et al.*, 1993). Additional potential herbal sources of new chemical entities for the management of Diabetes mellitus include *Coccinia indica* (Kimble *et al.*, 1996), *Gymnema sylvestre* (Baskaran *et al.*, 1990) and *Panax quinquefolius* (ginseng) (Sievenpiper *et al.*, 2004). Others include *Annona muricata* (Adewole and Caxton-Martins, 2006), *Hypoxis hemerocallidi* (Ojewole, 2006), *Vernonia amygdalina* (Ebong *et al.*, 2008) *Berberis lyceum* (Gulfra *et al.*, 2007) *Aloe vera* (Noor *et al.*, 2008), *Trichosanthes cucumerina* (Adeeyo *et al.*, 2008) and *Allium cepa* (Yusuf *et al.*, 2012).

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been reported dangerous to human health (Thomas and Wade, 2001). Therefore, there is an urgent need to search for novel antioxidants from natural sources, which could be used in medicine and additives to nutraceuticals (Thomas and Wade, 2001).

Furthermore, multi-herbal preparations have been reported to possess anti-diabetic activity in animal studies and patients. Kim *et al.*, (2007) showed polyherbal drug *hachimi-jio-gen* lowered blood glucose and protected against development of pancreatic fibrosis and oxidative damage in Otsuka lang- Evans Tokushima fatty rats and that *Dangnyosoko*, a chinese herbal medicine has beneficial effects on glycemia and glucosuria as well as on islet histopathology in hyperglycaemic rats. Moreover *et al.*, (2008) studied the antidiabetic effects of Diasansar (R); (a polyherbal formulation containing *Gymnema sylvestre*, *Withania somnifera* and *Azadirachta indica*) on streptozotocin and fructose-induced type Diabetic mellitus and showed herbs are promising anti-diabetic therapies. In view of the fact that emphasis is now on herbal therapy, searchlight is on herbs that are edible of which *Moringa* is qualified. Moreso, *Moringa* (Abdul karim *et al.*, 2005)

and *Mistletoe* (Duong *et al.*, 2003) leaves have abundance of antioxidants.

Some herbal mixtures containing mistletoe extracts and some other plant ingredients are used in treating one ailment or the other. These mixtures are Iscador, Mistletoe tea, Helixor, *Moringa* tea and viable herbal solutions.

The study is therefore aimed at investigating some ameliorative effects of *Mistletoe* and *Drum-stick* leaf extracts on STZ-induced Diabetes mellitus on the pancreatic histo-architecture and insulin profile.

MATERIALS AND METHODS

Collection of *Mistletoe (Loranthus micrathus)* Leaves

Mature fresh leaves of *Mistletoe* were collected from mahogany tree in the premises of Ladoke Akintola University of Technology, Ogbomoso, Oyo State. A sample of the collection was taken to the herbarium of Pure and Applied Biology Department, LAUTECH, Ogbomoso, Oyo State where it was authenticated with voucher number LHO.

Extraction of *Mistletoe (Loranthus micrathus)* Leaf

Fresh leaves of *Loranthus micrathus* were air-dried (under shade). The leaves were ground to coarse powder using an electric blender. The powdered sample of 300 g was soaked in 3000 mls of distilled water for 24 hours in a measuring cylinder. The mixture was homogenised in Explosion Proof Blender for 120 seconds, then filtered using a whatman filter. The filtrate in the round bottom flask was put on a heating mantle (Barnstead/ electrothermal) at 100°C for 7 hours. The concentrate formed was taken to an oven at 50°C for 1hour the final residue of 80 g was a dark green mass which was stored at room temperature 25°C.

Collection of *Drum-stick (Moringa oleifera)* Leaves

Mature fresh leaves of *Moringa oleifera* were harvested from LAUTECH farm at Ogbomoso, Oyo State. A sample of the collection was compared to the voucher specimen at the herbarium of Pure and Applied Biology Department, LAUTECH, Ogbomoso, Oyo State where it was authenticated with voucher number LHO 436.

Extraction of *Drum-stick (Moringa oleifera)* Leaves

Fresh leaves of *Moringa oleifera* were air-dried (under shade). The leaves were ground to coarse powder using an electric blender. The powdered sample of 200 g was soaked in 2000 mls of distilled water for 24 hours in a measuring cylinder. The mixture was homogenised in Explosion Proof Blender for 120 seconds, then filtered using a whatman filter. The filtrate in the round bottom flask was put on a heating mantle (Barnstead/ electrothermal) at 100°C for 7 hours. The concentrate formed was taken to an oven at 50°C for 1hour the final residue of 60 g was a dark green mass which was stored at room temperature 25°C.

Animal Breeding

Fifty four Wistar rats (*Rattus norvegicus*) of both sexes were used for these studies. The animals were between 6 to 10 weeks old (150-200 g). Animals were kept in cages (9 animals/cage) and housed in the animal holdings of the Department of Anatomy, Faculty of Basic Medical Sciences, Animal House, Olabisi Onabanjo University, Ikenne-Remo, Ogun State. The animals were exposed to 12 hours light, 12 hours darkness cycle at room temperature. They were maintained on animal feeds and allowed access to water and feeds freely (*ad libitum*).

Induction of Hyperglycaemia

Hyperglycaemia was induced in twenty seven overnight-fasted randomly selected rats by a single intraperitoneal administration of Streptozotocin at 70 mg/kg bw (Lal, Korner and Mastsuo 2000). Streptozotocin (STZ) was dissolved in citrate buffer (0.1M, pH 4.5) just prior to injection. Hyperglycemia was allowed to develop for 72hours (Lenzen, 2008). Animals with Fasting Blood Glucose \geq 250 mg/dl were considered hyperglycemic (Tende, Ezekiel, Dikko and Goji. 2011) and were included in this study. Control animals (n= 9) received a single intraperitoneal injection of 0.1M citrate buffer (1ml/kg bw; pH 4.5)

Experimental Design

Fifty four Wistar rats were divided randomly into six groups of nine animals each. Control group (G₁) was normoglycemic animals that received neither STZ, Mistletoe nor Moringa Leaf extracts, G₂ was diabetic group that received Mistletoe leaf extracts only, G₃ was diabetic group that received Moringa leaf extracts only, G₄ was diabetic group that received neither Mistletoe nor Moringa leaf extracts, G₅ was normoglycemic group that received Mistletoe leaf extracts only, G₆ was normoglycemic group that received Moringa leaf extracts only.

LD₅₀ of Mistletoe and Drum-stick

The LD₅₀ of aqueous leaf extracts of Mistletoe and Drum-stick were done in the department of pharmacology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The LD₅₀ of Mistletoe was 400 mg/kg body weight while that of Drum-stick was 2000 mg/kg body weight of the animals.

Mistletoe and Drum-stick Modes of Treatment

The dose of the aqueous extracts of *Moringa oleifera* used in these studies was supported by the report of (Tende *et al.*, 2011). Aqueous extract of *Moringa oleifera* was dissolved in physiological saline daily and was administered orally with use of cannula to a group of hyperglycaemic rats (n=9) at 200 mg/kg bw (at 9.00 – 10.00 a.m. each day) for a maximum period of six weeks. Also the dose of aqueous leaf extracts of Mistletoe used in this study was based on a pilot study. Aqueous extracts of Mistletoe was dissolved in physiological saline daily and administered orally with the use of cannula to a group of hyperglycaemic

rats(n=9) at 40 mg/kg bw (at 9.00 – 10.00 a.m.each day) for a maximum period of six weeks. Furthermore a separate group of normoglycemic rats (n=9) were administered 200 mg/kg bw of *Moringa oleifera* extracts and another group of normoglycemic rats (n=9) received 40 mg/kg bw of Mistletoe extracts. Control rats comprised a group of normoglycemic rats (n=9) that received neither STZ, Moringa nor Mistletoe extracts. These rats however received an intraperitoneal dose of 0.1M citrate buffer (pH 4.5) Nine hyperglycaemic rats which received neither Moringa nor Mistletoe extracts constituted the diabetic control group.

Measurement of Blood Glucose

Blood glucose was estimated in overnight fasted rats at 9:00 – 10:00 hours using Glucose oxidase method of one touch ultra 2 glucometer. Blood was obtained from the median caudal vein of the tail by snipping the tip of the tail. The blood glucose was monitored weekly from two weeks (acclimatisation period) before the induction of hyperglycaemia and for six weeks of treatment.

Measurement of Body Weight

Body weight of the rats were recorded two weeks (acclimatization period) prior to induction of hyperglycemia and on a daily basis during treatment for six weeks. Weight was taken with a weighing scale.

Measurement of Relative Pancreatic Weight

The relative pancreatic weight was recorded as the ratio of the pancreatic weight to that of the experimental rat using sensitive weighing balance (Sony F3G brand).

Measurement of Feed and Water Intake

The quantities of feed and water consumed on a daily basis were documented each morning (9.00 hours) prior to Drum-stick and Mistletoe treatment

Termination of Treatment

For the purpose of assessing the morphological and biochemical changes occurring in the different groups, the treatments were done for six (6) weeks. After six weeks the animals were sacrificed by euthanasia. Laparotomy was performed, the pancreas was harvested, rinsed in phosphate buffered solution and fixed in 10% formol saline for histological procedures namely; Haematoxylin and Eosin stain, Masson's Trichrome staining for pancreas to demonstrate degree of fibrosis and morphological changes and Von Giessen stain to demonstrate the type of connective tissue fibres present. A portion of pancreas was homogenized in phosphate buffer solution (pH 7.4) and refrigerated for estimation of oxidative stress markers (lipid hydroperoxide, GSH and SOD) and enzymes of glucose metabolism (LDH and G6PDH).

Estimation of Pancreatic Insulin

Levels of pancreatic insulin was estimated in homogenate of pancreas. Pancreatic insulin concentrations of each treatment group were estimated using Mercodia ultrasensitive rat insulin elisa kit

(Mercodia, Uppsala, Sweden) (Jackel *et al.*, 2004). The kit contains the following reagents.

Coated 96-well plate (mouse–monoclonal anti-insulin), Calibrator 0 (0.00 $\mu\text{g}/1$), 1 vial, 600 μl , Calibrator 1 (0.02 $\mu\text{g}/1$), 1 vial, 1000 μl , Calibrator 2 (0.05 $\mu\text{g}/1$), 1 vial, 1000 μl , Calibrator 3 (0.15 $\mu\text{g}/1$) 1 vial, 1000 μl , Calibrator 4 (0.35 $\mu\text{g}/1$) 1 vial, 1000 μl , Calibrator 5 (0.75 $\mu\text{g}/1$), 1 vial, 1000 μl , Calibrator 6 (2.00 $\mu\text{g}/1$), 1 vial, 1000 μl , Calibrator 7 (5.50 $\mu\text{g}/1$), 1 vial, 1000 μl , Enzyme conjugate 11x, 600 μl , Enzyme conjugate buffer, 6ml, Wash buffer 21x, 40, Substrate TMB, 22 ml, Stop solution, 0.5 M H_2SO_4 , 7 ml

Assay Procedure for Pancreatic Insulin

All Reagents were brought to room temperature and the assay was performed using the mouse a monoclonal anti-insulin-coated 96-well plate. Assay was performed as enumerated below:

- ✓ Wells were designated (in duplicate) as calibrators (standard curve) and samples (homogenate)
- ✓ 25 μl of calibrator 0 was added to each well (calibrator and sample well)
- ✓ 5 μl of each of calibrator 0 and calibrators 1-7 was added to the respective calibrator wells
- ✓ 5 μl of each of the samples (homogenate) was added to each of the sample wells
- ✓ Enzyme conjugate solution (50 μl) was then added to each of the wells (calibrator and sample wells)
- ✓ Incubation was done (with gentle mixing on a shaker) at room temperature (25 °C) for 2 hours, followed by repeated washing of the wells six times with 350 μl of the wash buffer. For each wash, the contents of the wells were completely aspirated. After the 6th wash, the multiwell plate was inverted and tapped firmly on an absorbent paper.
- ✓ After washing, 200 μl of substrate TMB was added to each well, followed by
 - Incubation for 30 minutes at room temperature (25^oc)
- ✓ The reaction was stopped by the addition of 50 μl of 0.5 M H_2SO_4 (stop solution) to each well. The reaction volume was mixed on a shaker for 5 seconds
- ✓ Absorbance of the coloured product was read at 450 nm in a microplate reader (ETI-System Fast Reader, SORIN Biomedica, Vicenza, Italy)
- ✓ A calibration curve was constructed (absorbance vs. insulin concentration of calibrators 1-7), and the concentrations of insulin in each sample volume was estimated. Results were expressed as μg insulin/L of homogenate.

Tissue Processing for Light Microscopy

Paraffin Wax embedding of the pancreas was done. At euthanasia, each organ was rinsed in PBS, trimmed free

of adipose tissue, cut into smaller pieces (3 mm x 3 mm) and fixed in 10% formalin, for a maximum of 48 hours. Dehydration of the sample was done in graded alcohol, clearing of the sample in two (2) changes of Xylene I and II, Wax Infiltration of the sample and Embedding (Inclusion) of the sample was done in paraffin wax. 5 μm -thick sections of the pancreas was cut on a Reichert-Jung 2050 rotary microtome (Cambridge Instruments, Germany). Sections were floated on the water bath at 50°C, and mounted on pre-washed, sterilized, 25.4 mm x 76.2mm glass slides (Pearls, China). Slides were washed with detergent, rinsed with deionised water, and sterilized with 70% ethanol. Mounted Paraffin sections of the pancreas were stained in Haematoxylin and Eosin, Masson's Trichrome Staining and Verhoeff Van Gieson Staining to demonstrate the type of connective (Bancroft and Stefen, 1982).

Photomicrography

Photomicrography of histological sections of the pancreas were taken with a Leica Microscope (Tokyo, Japan) coupled with desktop and CPU at Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Osun State.

Morphometric Analysis

Morphometric analysis of β -cell density in the islet of all the six groups was done using transparent grid. The slides were examined with light microscope, objective lens of power X40. The transparent grid was inserted into the eye piece of light microscope and the density of β -cells per mm^2 of the islet were counted.

Statistical Analysis

Descriptive and inferential statistics were carried out as follows: Data were analysed using Excel 2007 (Microsoft Corporation, U.S.A). Data were expressed as mean \pm standard error of the mean (mean \pm SEM). Mean values were compared using student t-test. P values less than 0.05 (P < 0.05) were taken to be statistically significant. All graphs were drawn with Excel 2007 and graph pad prism 5 (Microsoft Corporation U.S.A).

RESULTS

Blood Glucose on Weekly Basis (mg/dl)

Table 1 showed the blood glucose of different groups on weekly basis. The untreated diabetic group rats were hyperglycemic at week 0 (261.57 \pm 9.10 mg/dl) and remained so until the end of the sixth week (410.69 \pm 11.34 mg/dl). The value was significantly different from normal control (60.16 \pm 3.32 mg/dl) P < 0.05. Whereas the Mistletoe (260.12 \pm 8.18 mg/dl) and Drum-stick (264.68 \pm 8.57 mg/dl) treated Diabetic groups had high blood glucose at week 0 up to week 2 which were significantly different from control. P < 0.05. From the third week the blood glucose levels of Mistletoe (105.22 \pm 4.22 mg/dl) and Drum-stick (94.33 \pm 5.62 mg/dl) treated groups were comparable to normal control and not significantly different P > 0.05

Table 1: Blood Glucose on weekly basis. Data are expressed as Mean±SEM (P<0.05).

	WEE K -2	WEE K -1	WEE K 0	WEE K 1	WEEK 2	WEEK 3	WEE K 4	WEE K 5	WEE K 6
CONTROL (n=9)	89±4. 96	88.4± 3.60	90.4± 4.40	93.8± 3.93	82.6± 2.60	75.11± 4.37	85.63 ± 2.86	74.2 4±3. 96	60.16 ± 3.32
DIABETES+MISTLETOE n=9	72.6± 2.27	91.0± 4.43	260.1 2±8.1 8*	212.6 7±7.2 3*	130.78± 11.32	105.22± 4.22	90.47 ± 2.68	76.3 7±3. 47	61.76 ± 2.09
DIABETES+DRUM-STICK n=9	90.72 ± 3.20	88.31 ± 5.05	264.6 8±8.5 7*	184.2 2±8.5 6*	102.11± 5.27	94.33± 5.62	88.27 ± 4.17	68.3 4±3. 18	56.6± 2.73
DIABETES (n=9)	76.22 ± 3.62	79.00 ± 5.94	261.5 7±9.1 0*	278.4 4±13. 51*	259.44± 14.07*	300.67± 14.96*	388.1 4±11. 33*	391. 46±1 1.23*	410.6 9±11. 34*
MISTLETOE ALONE n=9	83.14 ± 1.71	92.23 ± 3.21	86.45 ± 1.74	91.36 ± 2.33	84.35± 2.42	78.69± 3.11	72.78 ± 2.21	69.4 5±3. 22	68.89 ± 3.35
DRUM-STICK ALONE n=9	80.62 ± 4.09	86.34 ± 3.80	93.24 ± 2.43	89.12 ± 3.07	76.34± 2.41	64.78± 2.04	62.67 ± 3.26	64.8 9±3. 50	58.59 ± 3.62

Average Body Weight on Weekly Basis (g)

Table 2 showed the weekly changes in body weight of animals in various groups. At the third week there was an increase of 29.4% in the average body weight of the animals in untreated diabetic group (213.33± 2.18 g), but after the third week their average body weight started declining and by the sixth week (190.0± 2.29 g) there was a decrease of 18% compared to the initial average body weight at week 0 (169.24±2.25 g) with no significant difference when compared to normal control P>0.05. In drum-stick treated diabetic group, there was an increase of 16.5% at third week (197.0± 2.58 g), but at sixth week (208.46±2.38 g) the increase was 23% when compared with their weight at week 0 (165.57±2.84 g) with no significant difference when compared to normal control P>0.05. Also, in mistletoe treated diabetic group there was an increase of 3% in the average body weight of animals at third week (169.11± 2.91 g) and an increase of 20.5% at sixth week (205.66±3.37 g) when compared to their average body weight at week 0 (158.0± 3.11 g) with no significant difference when compared to normal control (204.78±3.43 g) P>0.05.

Table 2: Average Body weight on weekly basis. Data expressed as Mean±SEM. P<0.05*

	WEE K -2	WEE K -1	WEE K 0	WEE K 1	WEEK 2	WEEK 3	WEE K 4	WEE K 5	WEE K 6
CONTROL (n=9)	0.52± 0.041	0.54± 0.044	0.54± 0.039	0.56± 0.046	0.9± 0.038	1.01± 0.039	1.01± 0.043	1.07± 0.042	1.05± 0.039
DIABETES+MISTLETOE n=9	0.48± 0.51	0.48± 0.053	0.47± 0.021	0.49± 0.037	1.01± 0.057	1.07± 0.061	1.03± 0.071	1.06± 0.051	1.04± 0.067
DIABETES+DRUM-STICK n=9	0.51± 0.041	0.5± 0.056	0.56± 0.043	0.59± 0.049	0.91± 0.089	0.87± 0.067	0.9± 0.076	1.06± 0.081	1.03± 0.074
DIABETES (n=9)	0.41± 0.053	0.41± 0.076	0.43± 0.068	0.44± 0.099	1.09± 0.019*	1.97± 0.097*	2.11± 0.16*	2.18± 0.21*	1.99± 0.19*
MISTLETOE ALONE n=9	0.52± 0.048	0.52± 0.57	0.54± 0.49	0.58± 0.64	0.76± 0.036	0.89± 0.071	1.04± 0.045	1.02± 0.061	1.03± 0.042
DRUM-STICK ALONE n=9	0.48± 0.038	0.5± 0.056	0.5± 0.036	0.51± 0.58	0.64± 0.047	0.78± 0.039	0.89± 0.066	0.97± 0.051	1.01± 0.049

Relative Pancreatic Weight (g)

The relative weight of the pancreas in various groups as shown in table 3. There was a significant decrease in pancreatic weight in untreated diabetic group (0.418±0.046 g) compared to normal control (0.803± 0.067 g) P<0.05. In mistletoe (0.593±0.071 g) and drum-stick (0.775±0.076 g) treated diabetic groups the relative pancreatic weight was not significantly different to normal control P>0.05.

Table 3: Relative pancreatic weight in grams. Data are expressed as Mean±SEM. P<0.05*

CONTROL n=9	DIABETES+ MISTLETOE n=9	DIABETES+ DRUM-STICK n=9	DIABETES n=9	MISTLETOE ALONE n=9	DRUM-STICK ALONE n=9
0.803± 0.067	0.593±0.071	0.775±0.076	0.418±0.046*	0.705±0.052	0.736±0.043

Feed Intake on Weekly Basis (g/g bw/d)

Table 4 showed feed intake in all the groups on weekly basis. In untreated diabetic group there was a gradual increase in feed intake from third week (1.09± 0.012 g/g bw/d), there was a 2.5 fold increase which moved to three folds increase at fourth week (1.35± 0.014 g/g bw/d). From fourth week, the feed consumption started declining in this group and at sixth week (1.26± 0.099 g/g bw/d) the increase was just about 2.5 fold which were all significantly different from normal control (0.79± 0.031 g/g bw/d) P<0.05. Whereas in the drum-stick treated diabetic group there was a 2 fold increase at third week (0.72± 0.039 g/g bw/d) which was maintained till the sixth week (0.71± 0.035 g/g bw/d) and was not significantly different to normal control P>0.05. Also in mistletoe treated group there was a 2 fold increase at third week (0.68± 0.032 g/g bw/d) which was maintained till sixth week (0.71± 0.043 g/g bw/d) and was not significantly different to normal control P>0.05*.

Table 4: Feed intake at approximately weekly basis. Data are expressed as Mean±SEM. P<0.05*

	WEE K -2	WEE K -1	WEE K 0	WEE K 1	WEEK 2	WEEK 3	WEE K 4	WEEK 5	WEE K 6
CONTROL (n=9)	0.29± 0.039	0.3± 0.031	0.3± 0.027	0.31± 0.031	0.59± 0.041	0.77± 0.0042	0.78± 0.047	0.79± 0.036	0.79± 0.031
DIABETES+MISTLETOE n=9	0.27± 0.036	0.29± 0.041	0.3± 0.027	0.32± 0.034	0.52± 0.051	0.68± 0.032	0.7± 0.029	0.72± 0.037	0.71± 0.043
DIABETES+DRUM-STICK n=9	0.3± 0.031	0.31± 0.024	0.29± 0.037	0.3± 0.033	0.56± 0.042	0.72± 0.039	0.74± 0.046	0.73± 0.026	0.71± 0.035
DIABETES (n=9)	0.26± 0.051	0.29± 0.032	0.31± 0.047	0.32± 0.065	0.77± 0.098	1.09± 0.012*	1.35± 0.014*	1.3± 0.010*	1.26± 0.099*
MISTLETOE ALONE n=9	0.25± 0.024	0.27± 0.031	0.28± 0.028	0.30± 0.036	0.56± 0.019	0.74± 0.042	0.78± 0.039	0.77± 0.061	0.76± 0.057
DRUM-STICK ALONE n=9	0.28± 0.026	0.29± 0.033	0.30± 0.041	0.31± 0.037	0.58± 0.012	0.76± 0.024	0.80± 0.032	0.80± 0.027	0.81± 0.036

Water Intake on Weekly Basis (ml/g bw/d)

At week 0, the water intake was comparable in all the animals, in untreated diabetic group, there was an increase in the water intake from week 1(0.44± 0.099 ml/g bw/d) and the increase was maintained till the sixth week (1.99± 0.19 ml/g bw/d), the increase in water intake was significantly different when compared to the normal control P<0.05, whereas the water intake in mistletoe (1.04± 0.067 ml/g bw/d) and drum-stick (1.03± 0.074 ml/g bw/d) treated groups was not significantly different to that of normal control (P>0.05) at the end of sixth week (1.05± 0.039 ml/g bw/d).

Table 5: Water intake on weekly basis. Data are expressed as Mean ±SEM. P<0.05*.

	WEE K -2	WEE K -1	WEE K 0	WEE K 1	WEEK 2	WEEK 3	WEE K 4	WEEK 5	WEE K 6
CONTROL (n=9)	0.52± 0.041	0.54± 0.044	0.54± 0.039	0.56± 0.046	0.9± 0.038	1.01± 0.039	1.01± 0.043	1.07± 0.042	1.05± 0.039
DIABETES+MISTLETOE n=9	0.48± 0.51	0.48± 0.053	0.47± 0.021	0.49± 0.037	1.01± 0.057	1.07± 0.061	1.03± 0.071	1.06± 0.051	1.04± 0.067
DIABETES+DRUM-STICK n=9	0.51± 0.041	0.5± 0.056	0.56± 0.043	0.59± 0.049	0.91± 0.089	0.87± 0.067	0.9± 0.076	1.06± 0.081	1.03± 0.074
DIABETES (n=9)	0.41± 0.053	0.41± 0.076	0.43± 0.068	0.44± 0.099	1.09± 0.019*	1.97± 0.097*	2.11± 0.16*	2.18± 0.21*	1.99± 0.19*
MISTLETOE ALONE n=9	0.52± 0.048	0.52± 0.57	0.54± 0.49	0.58± 0.64	0.76± 0.036	0.89± 0.071	1.04± 0.045	1.02± 0.061	1.03± 0.042
DRUM-STICK ALONE n=9	0.48± 0.038	0.5± 0.056	0.5± 0.036	0.51± 0.58	0.64± 0.047	0.78± 0.039	0.89± 0.066	0.97± 0.051	1.01± 0.049

Pancreatic Insulin Level ($\mu\text{g/L}$).

Pancreatic Insulin levels of all the six groups at the sixth week were shown in table 6. The Pancreatic Insulin levels of Mistletoe ($3.4\pm 0.186 \mu\text{g/L}$) and Drum-stick ($3.5\pm 0.088 \mu\text{g/L}$) treated hyperglycemic groups are not significantly different from normal control (3.8 ± 0.25

$\mu\text{g/L}$) $P>0.05$. But the Pancreatic Insulin level of the untreated hyperglycemic group ($1.5\pm 0.153 \mu\text{g/L}$) was low and decreased by 2folds compared to normal control. This decrease was significantly different from normal control rats $P<0.05$.

Table 6: Pancreatic Insulin Level. Data are expressed as Mean \pm SEM. $P<0.05$.*4.7 Biomarkers of Oxidative Stress.

	Control N=9	Diabetic+ Mistletoe N=9	Diabetic+ Drum- Stick N=9	Diabetic N=9	Mistletoe Alone N=9	Drum-Stick Alone N=9
Pancreatic Insulin Level	3.8 ± 0.25	3.4 ± 0.186	3.5 ± 0.088	$1.5\pm 0.153^*$	3.4 ± 0.033	3.9 ± 0.088

Morphometric Analysis

The number of viable β -cells per mm^2 of the Pancreatic Islet were measured using transparent grid at the end of this study at sixth week. The β cellular density per mm^2 of islet in Mistletoe (3.00 ± 0.32) and Drum-stick (4.00 ± 0.51) treated hyperglycemic groups were not significantly different to control (5.40 ± 0.51) $P>0.05$. Whereas in untreated hyperglycaemic group ($1.80\pm 0.37^*$), the β cellular density per mm^2 of islet was significantly different to control $P<0.05$.

Histological Observation

The light microscopy showed a disorganized cytoarchitecture and reduced cell mass in the Pancreatic Islet of untreated diabetic group (Plates 1D and 3D).

There was also some degree of fibrosis in this group (Plates 3D and 5D). The normal cytoarchitecture was maintained in the pancreatic islet of Drum-stick treated group with associated improved vascularization. There was also increase in β cell mass in this group (Plates 1C and 3C). There was slight distortion in the cytoarchitecture in Pancreatic islet of Mistletoe alone group (Plates 2A and 4A). There were some degree of disorganisation in the cytoarchitecture in the islet of Mistletoe treated hyperglycaemic group with slight reduction in the cell mass (Plates 3B). Elastic fibres were extensively deposited with high intensity in the untreated diabetic group. This deposition was even seen in the acini of this group (Plate 5D).

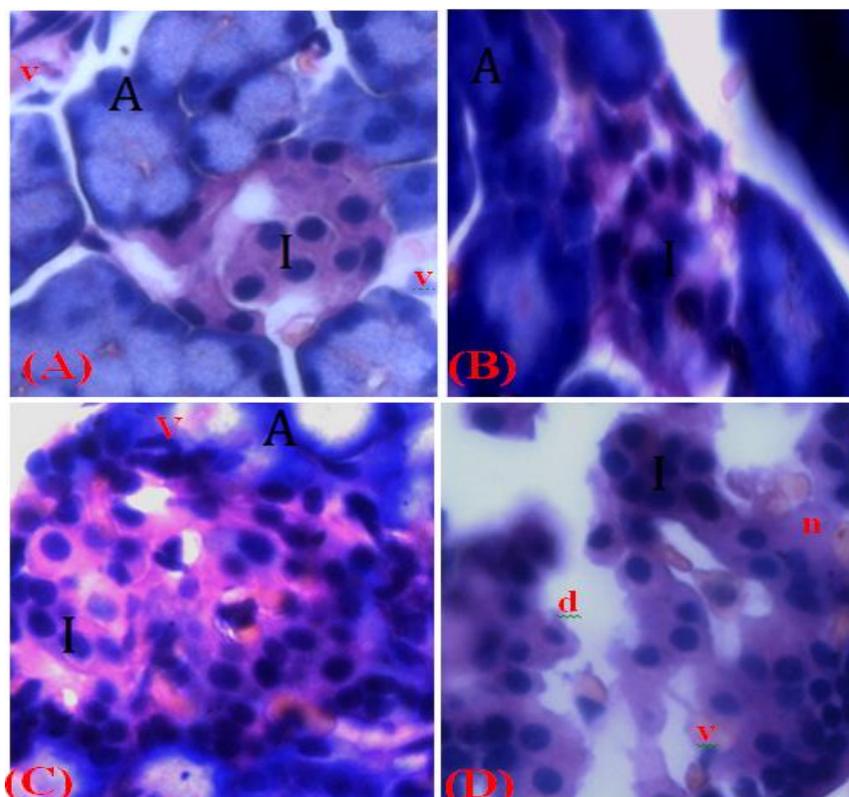


Plate 1: Photomicrograph showing the Pancreatic islet at day 42. H&E X1000. A-Normal control, B-Diabetic+Mistletoe, C - Diabetic+Drum-stick, D - Diabetic only. I- Pancreatic islet, A-Acini, v- Blood vessel, d- Disorganised cytoarchitecture of Islet and n- Fibrotic tissue.

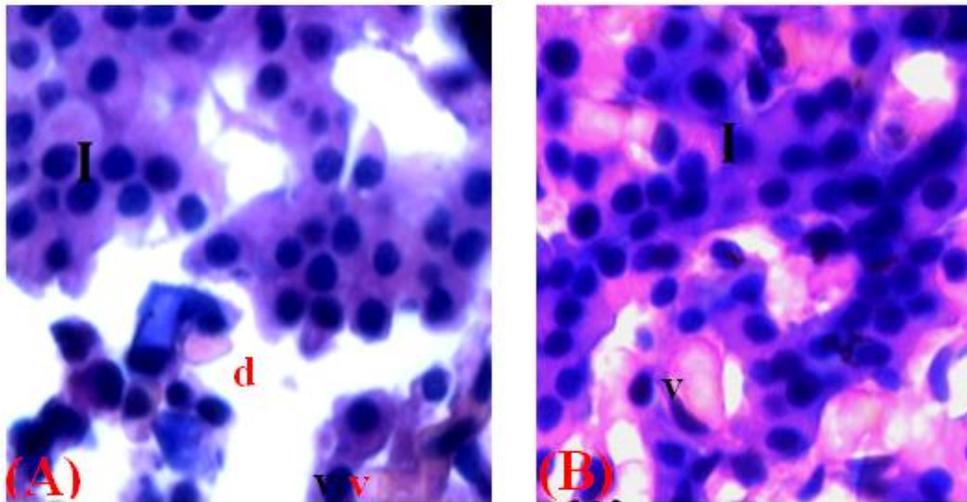


Plate 2: Photomicrograph showing the Pancreatic islet at day 42. H&E X400. A- Mistletoe only, B – Drum-stick only. I- Pancreatic islet, v- Blood vessel and d- Disorganised cytoarchitecture of Islet.

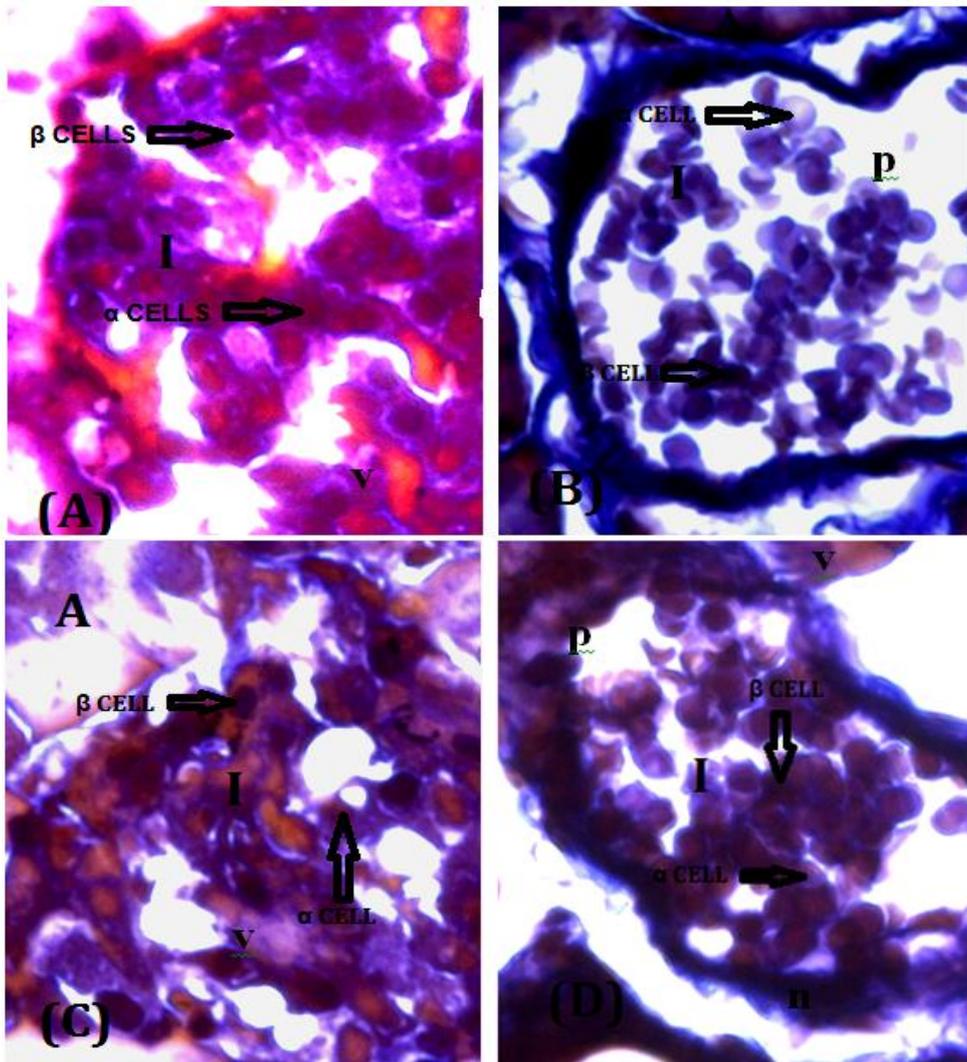


Plate 3: Photomicrograph showing the Pancreatic islet at day 42. Masson's Trichrome X1000. A- Normal control, B – Diabetic+Mistletoe, C – Diabetic+Drum-stick, D – Diabetic only. I- Pancreatic islet, A-Acini, v- Blood vessel, p- paucity of cell, n- Fibrotic tissue, α - cell and β -cell.

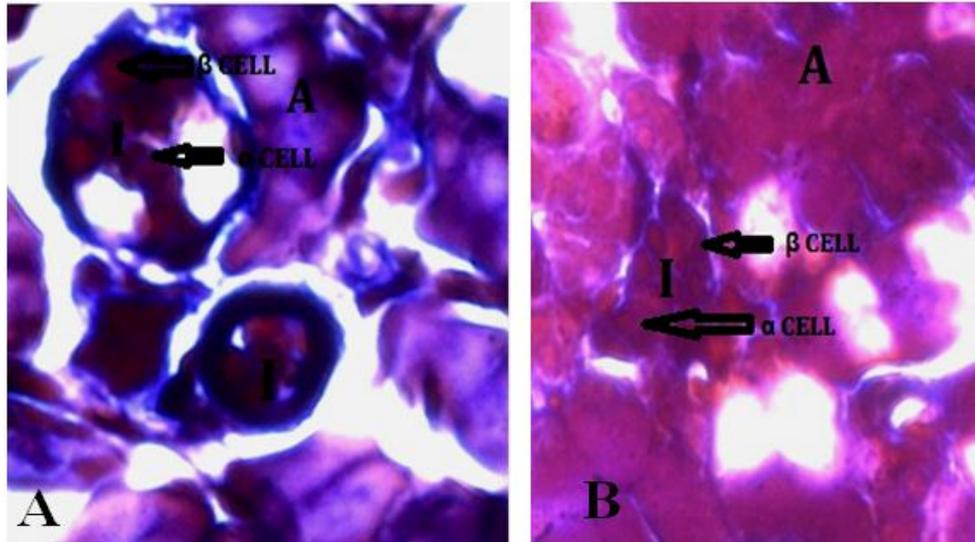


Plate 4: Photomicrograph showing the Pancreatic islet at day 42. Masson's Trichrome X1000. A- Mistletoe only, B – Drum-stick only. I- Pancreatic islet, A-Acini, α - cell and β -cell.

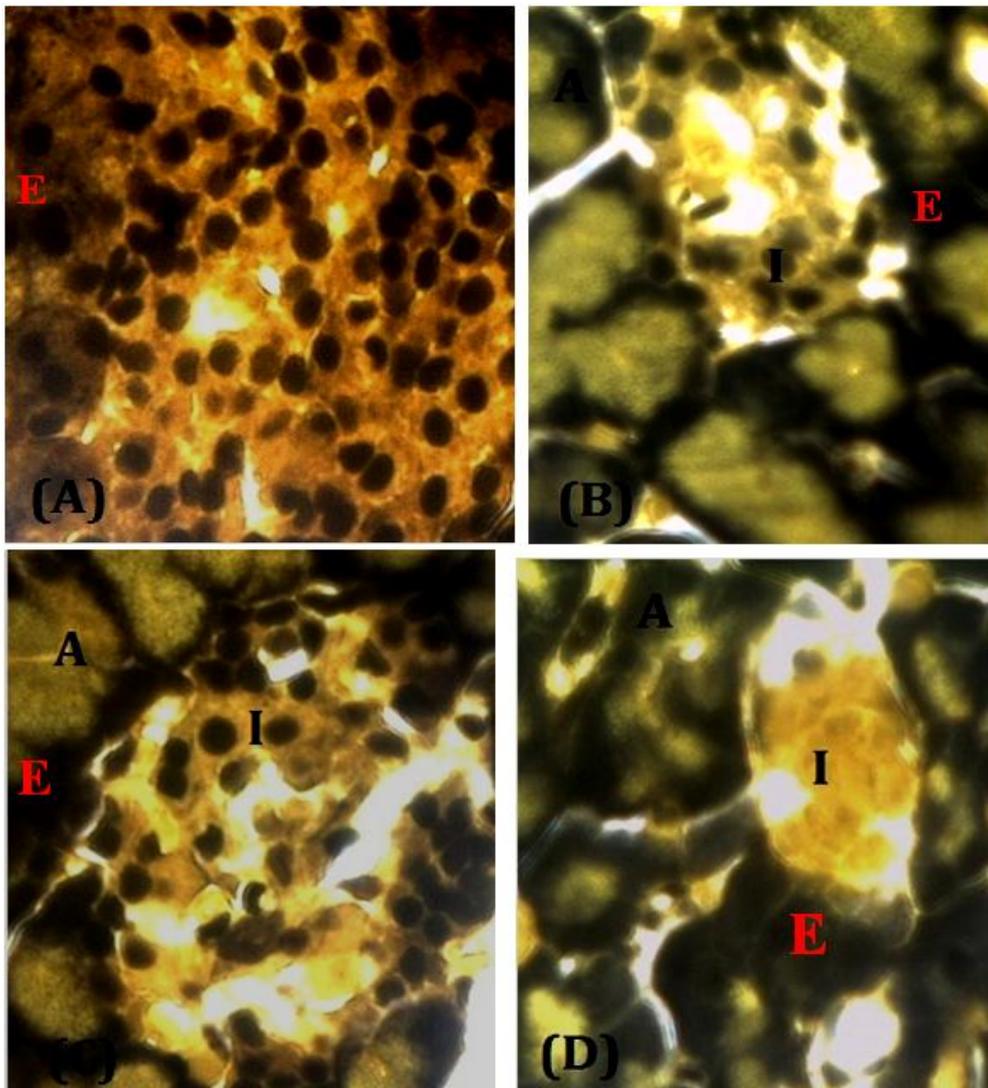


Plate 5: Photomicrograph showing the Pancreatic islet at day 42. Vorhoeff Van Gieson X1000. A- Normal control, B – Diabetic+Mistletoe, C – Diabetic+ Drum-stick, D – Diabetic only. I- Pancreatic islet, A-Acini and E – Elastic fibre.

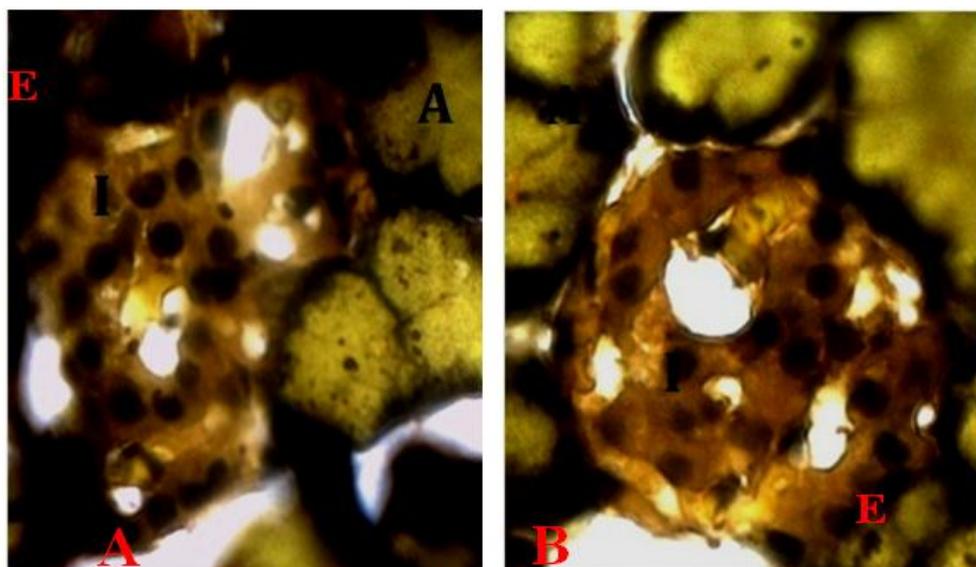


Plate 6: Photomicrograph showing the Pancreatic islet at day 42. Vorhoeff Van Gieson X1000. A- Mistletoe only, B – Drum-stick only. I- Pancreatic islet, A-Acini and E – Elastic fibre.

DISCUSSION

In this study, treatment of STZ induced hyperglycaemia rats with aqueous leaf extract of *Moringa oleifera*, at a dose of 200mg/kg/d, produced normoglycaemia in 88.9% of animals by the end of the first week of treatment and all the animals had become normoglycaemic by the end of the second week (Table 1). These findings when compared with hyperglycaemia animals treated with Mistletoe at 40mg/kgbw/d, showed a relative advantage because in Mistletoe treated animals, at the end of first week 77.8% were normoglycemic, 88.9% at the end of second week and 100% normoglycemia was achieved in these animals after the third week (Table 1). Hypoglycemia was achieved in these animals after the third week. Hyperglycemic activities of *Moringa* leaf had been reported by Tente *et al.* (2011), and that of Mistletoe was documented by Omeje *et al.*, (2013).

Hypoglycemic activities was achieved through accentuation of release of insulin from B cells of islets of langerhans of pancreas, prevention of uptake of glucose from GIT as seen in *alphagluconidase* or pancreatic amylase enzyme inhibitors, prevention of gluconeogenesis and glucogenolysis (Adewole *et al.*, 2007). *Moringa* hypoglycemic activities is reported due to the presence of alpha gluconidase and pancreatic amylase enzyme inhibitors (Shankar *et al.*, 2011). These enzyme inhibitors prevent the digestion of glucose into an absorbable product, hence the inability of blood glucose to increase after glucose intake. The presence of these inhibitors was reported in plants like *Morus alba*, which was able to exhibit hypoglycaemic activity (Sudha *et al.*, 2011). Also the hypoglycaemic activity of *Moringa* leaf extract is due to presence of antioxidants like flavonoids, phenol, vitamin C and E in it (Maxwell *et al.*, 2014). Duong *et al.*, (2003) reported that flavonoids and phenols are powerful hydrosoluble

antioxidants in biological fluids. The antioxidants are able to prevent further destruction of beta cells in the pancreatic islets. There was also an enhanced insulin secretion in Mistletoe and *Moringa* treated groups. Viable beta cells were able to produce sufficient insulin to prevent hyperglycemia in these groups. Destruction of beta cell by hyperglycemia is usually due to release of oxygen radicals and superoxides (Adewole *et al.* 2007). Presence of antioxidants is reported to prevent this destruction (Shankar *et al.*, 2011). Also, early attainment of normoglycemia by the second week by *Moringa* leaf extract prevented the destructive effect of hyperglycemia on beta cell.

At the end of sixth week, the photomicrographs (Plates 1D and 3D) of the untreated diabetic group showed necrosis of islet with paucity of cells and disorganised cytoarchitecture. The STZ given to these was taken up by β cells, the STZ caused the death of these cells by DNA fragmentation (Suwattanee *et al.*, 2014). In the hyperglycaemic group treated with Mistletoe and *Moringa* leaf extracts, the photomicrograph (Plates 1B, 3B, 1C and 3C) showed improvements in the morphology of the pancreas with increased number of viable β cells when compared with untreated hyperglycaemic group. The observed enhancement in the cellular density by these herbs was in contradiction to the work of Penard *et al.*, (2008) that β cells were post mitotic and were not capable of regeneration or undergo mitosis. However, the finding was in agreement with the observations of Nir *et al.*, (2007) and Meier *et al.*, (2006) that β cells still possessed regenerative potentials even in diabetic state. The real mechanism of improvement in islet morphology and cell mass in Mistletoe and *Moringa* treated groups are not clear, but the morphological improvement seen in these groups was absent in untreated diabetic group.

There was a widespread fibrotic degeneration of the pancreas in untreated diabetic rats (Plates 5D) in this study which agreed with the work of Hayden, (2007). He reported that Diabetes mellitus was associated with islet fibrosis. There were significant reduction in fibrotic deposits in the pancreas of Mistletoe and Moringa treated hyperglycaemic rats (Plates: 5B and 5C) in agreement with Kos *et al.*, (2004) who reported the prevention of pancreatic fibrosis by antihyperglycaemic therapies.

Hypoglycaemia activities of Mistletoe leaf extract were already documented to be due to presence of antioxidants which prevented destructive effect of hyperglycemia on beta cells (Adewole and Caxton Martins, 2006). It was also documented that Mistletoe has some insulin secreting activities (Omeje *et al.*, 2013). In the present study the relative weight of the pancreas of Moringa treated group increased significantly compared to the diabetic group, also the relative pancreatic weight of untreated diabetic group decreased significantly when compared to control group (Table 3). There was no significant difference in the relative weights of pancreas of Moringa treated group and control (Table 3). This is due to the nutrients in Moringa leaf such as protein which was reported to be twice that of milk, weight (Maxwell *et al.*, 2014) which served as an enhancing factor in weight of the organs of the animals.

Oxidative stress is a co-founding factor in Diabetes mellitus, and it contributes to the pathogenesis and complications (Ajit *et al.*, 2014) of the disease. Depletion of antioxidants increased the chances of developing complications in diabetes and intake of antioxidants improved the complication (Shankar *et al.*, 2011). Beta cells are vulnerable to oxidative insult in that they possess relatively poor complement of antioxidants (Shoichi *et al.*, 2012). In chronic hyperglycemia oxidative stress is induced via several mechanisms (Eiichi and Takeshi, 2010). STZ diabetes diabetes increases in pancreatic oxidative stress are associated with the progression of the disease (Giacco and Brownlee, 2010), as confirmed in this study. STZ is a nitric oxide (NO) donor; and NO had been reported to mediate the destruction of pancreatic islet cells, probably via DNA damage (Eiichi and Takeshi, 2010). In addition to NO, STZ also generate ROS (from the action of STZ on the mitochondria and from increased xanthine oxidase activity) (Szkudelski, 2001). Thus, scavenges of NO and ROS (Oseni and Idowu, 2014) have been reported to possess beneficial effects against DNA damage and beta cell toxicity induced by this substances. There is a growing body of evidence that oxygen derived free radicals are involved in the pathogenesis of over fifty human diseases (Moskovitz *et al.*, 2002).

Moringa contains vitamin C that is as much as that of 7 oranges in weight and beta carotene that is 4times of carrot weight (Maxwell *et al.*, 2014). These high

antioxidant level enhances the activities of Moringa compared to Mistletoe.

Although, current opinion on the regenerative capacity of β cells is rather conflicting. The long-held belief that these cells are post-mitotic had been challenged by recent findings. In mice, the work of Dor *et al.*, 2004 showed that terminally differentiated β cells retain a significant proliferative capacity in vivo. Also, Nir *et al.*, 2007 reported a similar finding in a murine model of diabetes. In vivo evidence that human β cells possess generative potential, even in the diabetic state had been reported by Meier *et al.*, 2006. However recent in vitro studies of Parnaud *et al.*, 2008b; on adult human β cells yielded contrary findings. The contrary findings above notwithstanding, the work of Parnaud *et al.*, 2008a reported that β cells could at least divide in rodents which is in agreement with the findings in this study.

The effects of the leaf extract on β cell proliferation may not be the only reason for normoglycemia. There are extra-islet stem cells from the liver, spleen, bone marrow and exocrine pancreas which can be responsible for normoglycemia (Meier *et al.*, 2006). Also it was documented that β cells were present in the spleen of Mascona snake (Adeeyo *et al.*, 2008), that means extra pancreatic islet cells could not be ruled out in other animals. The oleic acid present in Moringa also assist in the release of insulin from residual β cells and extra-pancreatic islet cell to produce normoglycemia. Also early attainment of normoglycemia by Mistletoe treated animals up-regulate G-6-PDH hence β cell proliferation seen. It is important to note that there was no improvement in islet morphology in untreated hyperglycaemic group, the mechanism of improved islet morphology and improved cell mass in Mistletoe and Moringa treated hyperglycaemic groups (more in Moringa than Mistletoe treated) are unclear. It is noteworthy that Adewole *et al.*, (2007) reported improved islet morphology in Mistletoe treated hyperglycaemic rat. The above findings are in agreement with what was seen in the present study where there is an improved islet morphology and enhancement of cell mass in Moringa and Mistletoe treated hyperglycaemic rat. In Moringa and Mistletoe treated groups, the pancreatic insulin level is similar to control (Table 6), due to improved islet cell mass. This study has provided morphologic evidence supporting the traditional uses of Mistletoe (Delioman *et al.*, 2002) and Moringa (Adedapo *et al.*, 2010) as antidiabetic therapy as well as use of Mistletoe in some antidiabetic preparations like Iscador and Moringa in Moringa tea.

CONCLUSION

Aqueous leaf extracts of Mistletoe and Moringa possess antihyperglycaemic effects and pose effective therapy in the management of hyperglycaemia and protect from the disabling effects of Diabetes mellitus by mopping up free oxygen and superoxide radicals. Enhancing as well the pancreatic insulin level.

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