The Role of an Aqueous Extract of Cactus on Histopancreatic Architecture in Streptozotocin Induced Diabetes Mellitus in Wistar Rats

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ABSTRACT

Herbs have been used as medicinal for several years to cure or rather manage different ailments such as diabetes mellitus. This is very common in rural settings of sub-saharan Africa and is now being adopted in urban areas. Opunta species have widely been seen to contain antihyperglycaemic effects. To evaluate the antihyperglycaemic and histopancreatic effect of aqueous extract of prickly pear cactus (Opuntia species) cladodes in streptozotocin-induced diabetic male Wistar rats in vivo.

This was a laboratory based research conducted at Mulungushi University involving 30 wistar rats (Rattus norvegicus) that weighed between 160-200 g which were randomly selected into five groups (6 per cage); normal control, diabetic control, diabetic +metformin, diabetic +cactus and cactus only group. Initial blood glucose levels were obtained from the tail and record then Diabetes mellitus was induced using a single intraperitoneal dose of streptozotocin (70mg/kg BW) and established a persistent state of hyperglycemia after 72hours. The aqueous cactus extract of 100 mg/kg BW and metformin of 100 mg/kg BW was administered orally using intragastric cannula daily for a period of 4 weeks.

At the end of the fourth week, Diabetes + cactus and diabetes + metformin groups body weights were statistically significant when compared to the diabetic group (P<0.05). While diabetic group was statistically significant when compared to normal control (P<0.05). The relative weight of the pancreas in diabetic group was statistically significant to other groups (P<0.05). The blood glucose in diabetic + cactus was normoglycaemic at 3rd week, Diabetic + metformin group when compared to diabetic group was statistically significant (P<0.05). The normal control and cactus only groups maintained normoglycaemic till the end of the 4th week. The histological findings of the normal control and cactus only group showed normal pancreatic cytoarchitecture. Diabetes group showed high degree of disorganization in the cytoarchitecture in the islet with reduction in β cell mass and deposition of elastic fibres. The Diabetic+Metformin group showed slight decrease in the cell mass (β cells) and elastic fibres were extensively deposited. Diabetic+Cactus treated group exhibited normal histology of the pancreas with increased number of β cells.

Opuntia species are able to lower elevated blood glucose levels and ameliorate the effects of STZ on the pancreatic morphology.

Keywords: Cactus, diabetes mellitus, pancreas, streptozotocin, Wistar rat.
I. INTRODUCTION

Diabetes Mellitus has long been regarded as a chronic metabolic disorder and a public health problem in the United States and around the world characterized by persistent hyperglycemia. Managing diabetes mellitus is really important because of the devastating complication that occur if poorly controlled. However, it is costly to manage many patients find it a challenge to keep up with the prescribed medicines and advised diet [1].

Diabetes mellitus is defined as persistent hyperglycemia measured on two or more occasions occurring as a result of impaired insulin secretion, insulin action or both [2], [3]. Type 1 diabetes, also known as insulin-dependent diabetes mellitus, is caused by insufficient or non-existent insulin output and accounts for around 10% of all cases worldwide. It is triggered by the autoimmune destruction of beta pancreatic islet cells, resulting in complete insulin deficiency.

Type 2 diabetes, also known as non-insulin-dependent diabetes mellitus with relative insulin deficiency, develops as a result of peripheral insulin resistance and insufficient compensatory secretion, accounting for roughly 90% of all cases worldwide [4].

Traditional medicine has been practiced for many years with so many plant extracts being used for the treatment or management of many ailments of which Diabetes mellitus is not an exception. Various plants have been studied for their hypoglycemic effects in both animal and human studies with some being sold on the markets such as Chinese herbal medicine. Most herbal medicines have not been evaluated for their potential benefits or adverse effects on the body tissue and organs despite the fact that these therapies have shown potential efficacy. This is because they are not tested and poorly monitored if even done [5]-[7].

Cactus is a genus of plants in the Cactaceaeae family with a large number of species that have been studied for their nutritional and medicinal (as herbal) properties in various parts of the world. Opuntia is a cactus subspecies native to Mexico, but it can also be found in arid and semi-arid climates in tropical and subtropical regions of Latin America, Africa, and the Mediterranean [8]. It is known by its classic prickly pear, joined stems (cladodes), flattened pads, and edible fruits, which were introduced to Africa by exotic seeds. Opuntia stricta (nopal cactus or Opuntia ficus indica), also known as prickly pear cactus, is a dicotyledonous angiosperm plant that grows in parts of Sub-Saharan Africa, including Zambia, where the fruits and cladodes are eaten [8]-[10].

Studies have shown that it contains antioxidant and anti-inflammatory properties enabled by its constituent compounds as well as hypoglycemic and hypolipidemic effects thus being used as traditional medicine in the management of diabetes mellitus type 2, cardiovascular diseases and other ailments [9].

II. MATERIALS AND METHOD

A. Plant Extraction

Prickly pear cactus was obtained from Ellen brittke township of Livingstone city and was extracted as reported by [9]. The fresh leaves (cladodes) of the plant were cut into smaller pieces air dried under shade for one week then blended. The powder was soaked in water distilled water overnight.

B. Animals and Animal Management

Healthy adult male wistar rats were used for this research weighing between 160-200 g and kept in 5 cages (6 per cage) in the Animal house of Department of Human Anatomy, Mulungushi University School of Medicine and Health Sciences. They were kept at standard conditions and acclimatized for 1 week and fed with standard animal feeds (pellets) and allowed access to clean water (ad libitum).

C. Induction of Diabetes

The animals were acclimatized for one week and blood glucose levels were measured on day one and day seven. Animals were fasted overnight (18:00-06:00 hours) and fasting blood glucose was measured using a glucometer before induction of hyperglycemia. Streptozotocin at 70 mg/kg bw [11] was administered as a single intraperitoneal dose to 18 rats, subjected to standard feeds and clean water.

The animals were monitored over 72 hours and fasting blood glucose measured. The fasting blood glucose greater than 7 mmol/L was considered hyperglycemic.

D. Experimental Design

This was a laboratory-based study, randomized control trial complete block. The animals were treated with standard care and all groups received the same amounts of feeds and water. A total of 30 Wistar rats were randomly assigned to different experimental groups as follows:

Group 1 (n = 6): Received sterile water orally once daily (Normal Control Group).

Group 2 (n = 6): streptozotocin-induced diabetic (diabetic control).

Group 3 (n = 6): diabetic and treated with cactus aqueous extract of 100mg/kg body weight orally once daily (Diabetic + cactus).

Group 4 (n = 6): diabetic and treated with metformin of 100 mg/kg body weight orally once daily (Diabetes + metformin).

Group 5 (n = 6): received cactus aqueous extract of 100 mg/kg body weight orally once daily (cactus only).

E. Cactus and Metformin Mode of Administration

The standard drug metformin was dissolved in physiological saline and administered orally to group 4 at a dose of 100 mg/kg bw. The cactus aqueous extract was also dissolved in physiological saline and administered using orogastric cannula to group 3 and group 5 (n = 6) at 100 mg/kg/bw daily (08:00 – 10:00 am) for a maximum of four week after induction.

F. Measurement of Blood Glucose

Blood glucose levels were obtained using a glucometer (Accu-Chek Compact Plus). Blood was collected from the median caudal vein of the tail by snipping the tip of the tail on a weekly base. The average blood glucose was obtained and recorded [12].

G. Measurement of Body Weight and Relative Organ Weight

The body weight of the animals were recorded in the first week of acclimatization and later on a weekly base.
throughout the period of treatment. The relative weight of the Pancreas was recorded after animal sacrifice [12].

H. Animal Sacrifice

The animals were sacrificed at the end of four week by cervical dislocation. Laparotomy was performed and Pancreas was harvested for study. The pancreas was rinsed and placed in formal saline over 72 hours for fixation. The harvested pancreas was processed for histological analysis using Haematoxylin and Eosin, Gomori and Von Giessen stain.

I. Statistical Analysis

Data analysis was done using Excel. It was expressed as mean ± standard error of mean (mean ± SEM). Mean values were compared using one way ANOVA. P values less than 0.05 were taken to be statistically significant.

J. Ethical Considerations

Ethics approval of the study protocol was granted by Mulungushi University School of Medicine and Health Science Research Ethics Committee (MUSoMHS-REC) (SMHS-MU2,2021-28). Standard requirements for the conduct of experiments on whole animals including the practice of good animal welfare and husbandry was adhered to in line with the laboratory standard operating procedures set by International Animal Care and Use Committee of Biotechnology Research Institute.

III. RESULTS

A. Average Body Weight on Weekly (g) Basis

Fig. 1 shows the body weight of the rats on a weekly basis. In the week of acclimatization (-1), there was no significance in boy weight among the groups. After induction and initiation of treatment in the second week, the weight didn’t change as much in other groups compared to the control. By week three of treatment there was a reduction in weight of rats in diabetic control (p<0.05) compared to those treated with cactus and metformin (p>0.05). The organ weight was lower than the diabetes cactus (p<0.05), as well as that of metformin group. However, there was no significant change between the diabetes control and those treated with metformin (p>0.05).

B. Average Blood Glucose on Weekly (mmol/L) Basis

Fig. 2 shows the average random blood glucose weekly in mmol/L. From week one to week four, there was no change in blood glucose for the control and cactus only group. After induction and initiation of treatment, increased levels of blood glucose were noticed in diabetic groups (p<0.05), but it reduced significantly in those treated with cactus by week three compared to the metformin group and diabetic control. By the end of treatment (28 days), 92.4% of the animals treated with cactus extract returned blood glucose levels to normoglycemic when compared to the metformin group were 70%.

C. Relative Organ Weight

Fig. 3 shows a graph of the average weight of the pancreas. The organ weight in those from the control group was higher than those of the diabetes control and treated but lower than the cactus only group (p<0.05). The diabetes control group organ weight was lower than the diabetes cactus (p<0.05), as well as that of metformin group. However, there was no significant change between the diabetes control and those treated with metformin (p>0.05).

D. Histological Findings

The cytoarchitecture of the pancreas in all groups was studied with different stains. The light microscopy showed normal cytoarchitecture with lots of cells in the Pancreatic islets of normal control and the cactus only groups (Fig. 4A, 5A, 6A); there was reduced cell mass in the Pancreatic Islet of the diabetic control group (Fig. 4B, 5B, 6B) some degree of disorganization and necrosis in the cytoarchitecture of diabetic group and Metformin treated group with slight reduction in the cell mass (Fig. 6B, and 6D). The normal cytoarchitecture was maintained in the Pancreatic islet of Cactus and Metformin treated groups (Fig. 4C and 4D, 5C and 5D, 6C and 6D) with an increase in β cell mass in (Fig. 4C and 4D). Elastic fibres were extensively deposited with high intensity in the diabetic group and Metformin treated group. This deposition was even seen in the acini of these groups (Fig. 6B and 6D).
Fig. 2. Average blood glucose on weekly basis (mmol/L). Data were analyzed using mean ± SEM and *p<0.05* considered significant.

Fig. 3. Relative pancreatic weight (g) on weekly basis. Data were analyzed using mean ± SEM and *p<0.05* considered significant.

Fig. 4. Photomicrograph showing the Pancreatic islet at day 28, H&E X400. A) Normal control, B) Diabetic, C) Diabetic+Cactus, D) Diabetic+Metformin and E) Cactus only. 1) Pancreatic islet, a) Acini.
IV. DISCUSSION

Diabetes mellitus is a chronic metabolic disease characterized by a state of persistent hyperglycemia and it was induced using streptozotocin (dose) in rats. A number of medicinal plants have been evaluated for antihyperglycemic effects or alleviation of physical and histological effects in animal studies. The animals were withdrawn from food and water for 12 hours before induction and their fasting blood glucose was tested. After 72 hours, random blood glucose was tested in the three groups induced (G2, G3, and G4). Treatment commenced for a period of four weeks with the standard treatment diethylbeganide (metformin) and Opuntia stricta cladode aqueous extract. All the groups induced with streptozotocin showed raised blood glucose levels in 96% of the animals (p<0.05) after 72 hours from the baseline (G2 DIABETIC CONTROL, G3 DIABETIC+CACTUS and G4 DIABETES + METFORMIN).

There was a reduction in blood glucose levels of the groups treated with cactus (at dose of 100 mg/kg bw in) and metformin after one week of treatment. By week four, a significant reduction in blood glucose was observed (p<0.05) in those treated with cactus and metformin groups compared to the diabetic control group. This finding demonstrated that aqueous extract of cactus contains antihyperglycemic effects in diabetic rats as in similar reported studies by [10].

According to [9] Opuntia stricta growing in Zambia was found to contain alkaloids, flavonoids, saponins, sterols,
carbohydrates (polysaccharides), phenols and tannins. It has also been found that Opuntia species contain dietary fibre which shows properties of reducing glucose levels by inhibiting intestinal glucose absorption as reported by [9]. The body weight of the animals obtained was between 160-200 g. Significant reduction in body weight (P<0.05) was observed in all the diabetic groups (G2, G3, G4) after one week of induction compared to those in control (G1), and cactus only (G5), also reported in other studies [13], [14]. This has been seen to result from the disturbed metabolism of fat, protein and carbohydrates due to oxidative stress. Increased breakdown of fat and proteins caused weight loss, especially in the diabetic control group. The weight significantly decreased throughout the period of treatment in the diabetic control but was restored in those treated with metformin and nearly to normal values in those treated with cactus extract by the third week which could be due to better glycemic control [15]. As reported by [16], aqueous cactus extract alleviates the oxidative stress caused by streptozotocin, thus, decreasing in weight loss of the rats treated. Reported studies show similar findings and showed that administration of cactus extracts altered physiological and histological effects due to streptozotocin. By the end of week four of treatment, the histological analysis revealed a normal cytoarchitecture of the pancreas in the normal control (Fig. 4A, 5A and 6A) as well as the cactus only group (Fig. 4E, 5E, and 6E). This finding demonstrated that cactus extract had little or no pathological effects on the pancreas of non-diabetic wistar rats. In the diabetic control group, necrosis of islets and disorganized cytoarchitecture were noticed. Streptozotocin lead to cell death by DNA fragmentation (Fig. 4B, 5B and 6B) due to oxidative stress and release of oxygen radicals and superoxide which also cause islet fibrosis as reported by [17]. There was an improvement in the pancreatic morphology in the group treated with cactus (Fig. 4C, 5C and 6C) and metformin (Fig. 4D, 5D and 6D) with an increase in number of viable β cells compared to that of diabetic control, this might due to the antioxidants flavonoids and phenols presence that have been reported to be hydrosoluble in this might due to the antioxidants flavonoids and phenols number of viable β cells compared to that of diabetic control, but was restored in th...