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Histological investigation of aqueous extract of cactus on the heart of diabetic Wistar rats

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Abstract

This study aimed to examine the effects of aqueous leaf extract of Cactus on the Heart of Diabetic Wistar Rats.

Thirty adult male Wistar rats weighing between (160-200) g were randomly grouped into five consisting of six rats each *viz*: A. normal control, B. diabetic only, C. diabetic treated with *Cactus,* and D. diabetes treated with Metformin only, and E. Cactus extract only. Diabetes was induced by a single intraperitoneal injection of Streptozotocin of 70mg/kg/body weight. After 72 hours of uninterrupted diabetes (blood glucose≥ 7 mmol), aqueous extracts of *Cactus and Metformin* were administered orally at 100 mg/kg body weight daily for four weeks and blood glucose level was recorded weekly. After four weeks of extract administration, animals were sacrificed by euthanasia. Organs were weighed and their weight was recorded. Data were analyzed using excel and one-way ANOVA.

The findings of this study showed that the blood glucose level of Diabetic +*Cactus* group and diabetes + Metformin were significant when compared to the diabetic group ($p<0.05$). The body weight and relative organ weight of the Diabetic +*Cactus* group and diabetes+ Metformin when compared to the diabetic group was significant $P<0.05$. Histological findings showed a normal control group had normal histoarchitecture with numerous healthy cardiomyocytes and normal distribution of collagen. Diabetic groups showed many degenerating cardiomyocytes and numerous depositions of collagen. Diabetes +Cactus and Metformin treated groups showed little disruption.

Aqueous extract of *Cactus* showed ameliorative effects on the heart of adult male Wistar rats against the damage initiated by diabetes.

Keywords: Streptozotocin; Heart; Cactus; Diabetic; Wistar rat

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1. Introduction

Diabetes mellitus is a chronic life threatening non-communicable devastating metabolic disorder which disrupts the control of glucose (blood sugar) in the body either by developing resistance or decreasing insulin production (1). Further, insulin is the primary hormone that regulates blood sugar levels and it is released by the Beta cells of the pancreas so as for it to allow the sugar to enter from the bloodstream into other cells by acting on insulin receptors. (2).

Diabetes mellitus can be classified in several ways based on cause and clinical presentation (3). Thus, there are 2 main types of diabetes mellitus namely type 1

diabetes mellitus and type 2 diabetes mellitus (T2DM) others include gestational diabetes mellitus and other specific types (4). Type 1 diabetes mellitus is autoimmune diseases accounting for about 5%- 10% of all cases of diabetes and is the most typical subtype diagnosed in patients younger than 20 years of age.(5).

However, Type 2 diabetes is caused by a both peripheral resistance to insulin action and an inadequate secretory response by the pancreatic β cells and accounts for about 90% to 95% of diabetic patients (6). Although classically considered "adult-onset," the prevalence of type 2 diabetes in children and adolescents has been increasing at an alarming pace because of increasing rates of obesity in these age groups (4).

Hyperglycemia is a sign of diabetes mellitus occurring as sequelae of deficient pancreatic insulin secretion due to pancreatic β cell destruction or poor insulin-directed mobilization of glucose by target cells over a prolonged period of time that results in classic triad of polydipsia (increased thirst), polyphagia (increased hunger) and polyuria (frequent urination) (7).

Diabetes mellitus if left untreated cause severe complication like, nephropathies, neuropathies ,retinopathies(blindness) organ failure, hypertension ,stroke, cardiovascular disease , progressive metabolic complication like diabetes ketoacidosis, nonketotic and hyperosmolar coma that can ultimately lead to premature death (8).

Cactus also known as nopal belongs to genus Opuntia which has been used for traditional practice as a medical herbal plant to control a variety of human illness this include diabetes mellitus (9). It is a plant which is part of the diet in Mexican and Mexican-American cultures (10).

2. Material and methods

The Cactus was harvested from Livingstone district, Southern Province of Zambia. It was subjected to identification at the University Of Zambia School Of Natural Sciences under the Department of Biological Sciences before the study began. The Cactus was air dried and pounded. The dry pounded Cactus was then ground and sieved to obtain a homogenous powder. The extraction was done using (11, 12) methods.

2.1. Animals and animal management

Thirty adult presumably healthy male Wistar rats (Rattus norvegicus) were used for this study. The animals were between 8 to 10 weeks old; body weight (160-200 g). Animals were kept in five cages (6 rats per cage) and housed in the animal holdings of the Department of Anatomy, Mulungushi University School of Medicine and Health Sciences. They were maintained on standard animal feeds (Wealth-gate pelletized feeds) and allowed access to clean water and feeds freely.

2.2. Induction of diabetes

Streptozotocin was used to induce diabetes. Rats were weighed, and a baseline glucose level was established after the overnight fasting period .The animals were injected with Streptozotocin calculated at a dose of 100 mg/kg body weight and reintroduced to the normal feeding cycle (13,14). It took about 72 hours for diabetes to be established in the animals following the administration of Streptozotocin therefore a fasting blood sugar was collected to determine the establishment of diabetes using the tail vein puncture 72 hours after administration of Streptozotocin. A glucometer was used to access blood glucose levels. Animals were considered diabetic with fasting blood glucose levels above 7 mmol/l.

2.3. Experimental design

Thirty adult healthy male Wistar rats were divided into five groups of 6 Wistar rats each. Control Group A was normoglycemia animals that received neither STZ nor Cactus extract , Group B was diabetic that did not receive neither STZ nor Cactus extract, Group C was diabetes treated with Cactus extract that received cactus extract only, Group D was diabetes treated with Metformin that received Metformin only and Group D was cactus extract only (15).

2.4. Cactus mode of administration

The dose of the aqueous extracts of Cactus used in these studies was adopted from the report (Saad *et al*.,2017). Cactus was dissolved in physiological saline daily and was administered orally with use of oro-gastric cannula to Group C rats (n=6) at 100 mg/kg bw of cactus extract (at 9.00 – 10.00 a.m. each day) for a maximum period of four weeks, Group D (n=6) at 100 mg/kg bw of metformin, Group E rats (n=6) were administered 100 mg/kg bw of cactus extracts. Group A rats (n=10) received neither STZ nor cactus extract (15).

2.5. Measurement of blood glucose

The blood glucose was evaluated in overnight fasted rats at 9:00 – 10:00 hours using Glucose oxidase method of one touch ultra 2 glucometer (Accu-Chek Compact Plus). Blood was obtained from the median caudal vein of the tail by snipping the tip of the tail. The blood glucose level was monitored weekly from two weeks (acclimatization period) before the induction of Diabetes and for four weeks of treatment (15, 16)

2.6. Measurement of the body weight (g)

Body weight (g) of the rats was recorded for two weeks (acclimatization period) prior to induction of diabetes and on a weekly basis during the experimental treatment for a period of four weeks. Body Weight was taken with a weighing scale (Venus VT 30 SL); (16, 17).

2.7. The relative organ weight (%)

The relative organ weight of the rat was evaluated as the ratio of respective weight of the brain and the terminal body weight of the same rat, the unit was recorded as percentage (%) using sensitive weighing balance (SonyF3G brand); (15, 17).

2.8. Histological process

At the end of the study, animals were sacrificed by euthanasia. They were laid supine on the dissecting board and pinned through the fore and hind paws. The skulls of the animals were dissected with bone forceps and each organ was carefully removed and weighed. The tissue for histological studies was fixed in freshly prepared formalin for 72 hours and processed for routine histological examinations stained with Haematoxylin and Eosin (H&E) to observe changes in the cellular morphology. Masson trichome stains was used for change in collagen.

2.9. Photomicrography

Photomicrography of histological sections of the prefrontal cortices will be taken with an Olympus Microscope (New York, United State of America) coupled with camera at Department of Human Anatomy, Mulungushi University School of Medicine and Health Sciences, Livingstone Campus, Zambia (17)

2.10. Statistical analysis

Data was presented as mean ± standard error of the mean (mean±SEM); analyzed using one way ANOVA and all graphs were drawn using Excel (Microsoft Corporation, U.S.A). P values less than 0.05 ($p<0.05$) was taken to be statistically significant.

3. Results

3.1. Average body weight on weekly (g)

Figure 1 shows changes in body weight of Wistar rats belonging to different groups on a weekly basis .After induction (week 0), the body weight of Wistar rats was measured and were normal without significant change when compared to the control group. In week 3 there was significant decrease in the diabetic group when compared to the control, diabetic+ cactus and cactus only groups which are significant ($p<0.005$). In week 4 diabetic group and diabetic+

Metformin continue showing a decline in body weight while the control, cactus only and diabetes+ cactus are still significant to diabetic group.

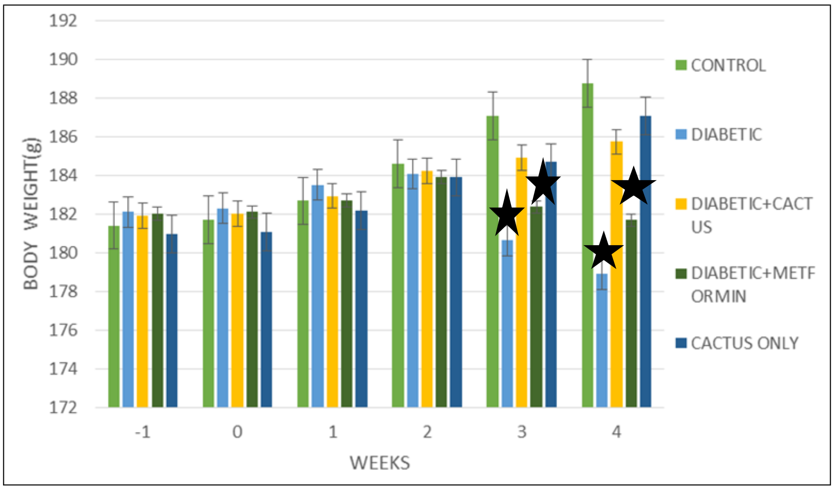

Figure 1 Histogram chart of average body weight on weekly basis. Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

3.2. Average blood glucose on weekly basis (mmol/L)

Figure 2 shows blood glucose levels which were taken weekly in various groups of Wistar rats. In the acclimatization week the blood glucose of various groups were normal with no significant difference when compared to control group. There was increase in blood glucose during the week of induction (week 0) in diabetes, diabetes + cactus and diabetes + Metformin. After treatment in week 1 diabetes + cactus showed a significant decline when compared to diabetes and diabetes+ Metformin which was significant. Diabetic group was still significant (p<0.05) to other groups up to the fourth week of treatment.

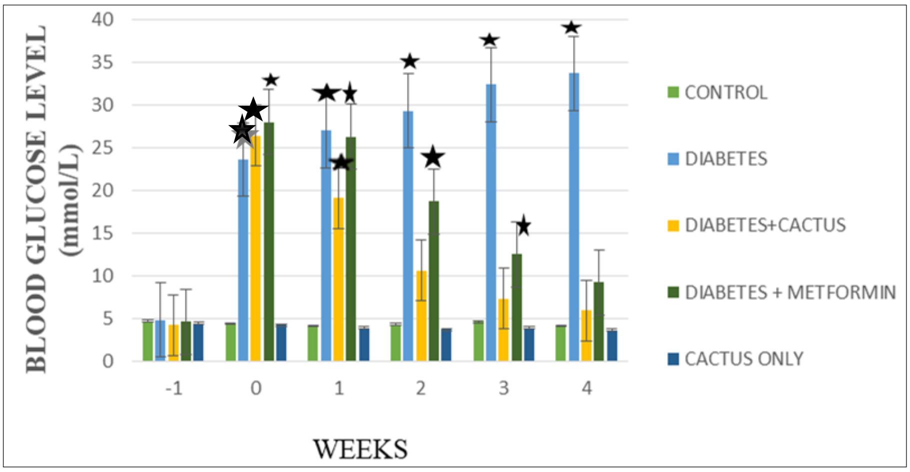

Figure 2 Histogram chart of blood glucose of Wistar rats on weekly basis. Data were expressed as mean ± SEM (p<0.05) * asterisk means significance at p<0.05

3.3. Relative organ weight

Figure 3 shows the relative weight of the heart in different groups of Wistar rats. The diabetes group showed a significant decline in heart weight as compared to control. Diabetes+ Cactus declined as compared to the control and cactus only but not significant (P>0.05). Diabetes +Metformin also showed decrease in heart weight when compared to control also not significant (P>0.05).

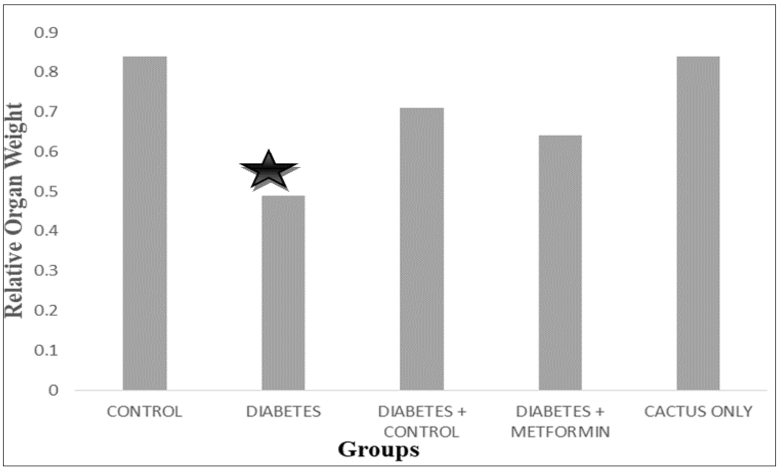

Figure 3 Bar chart of relative heart weight (g). Data were expressed as mean ± SEM ($p<0.05$) * asterisk means significance at $p<0.05$

3.4. Histology of heart

The heart in the normal control group showed normal histoarchitecture with numerous healthy cardiomyocyte, (Figure 4 A). Diabetic group showed that the histoarchitecture was disrupted with many degenerating cardiomyocyte (Figure 4 B). Cactus + metformin treated groups showed little disruption in their histoarchitectures and there are both healthy and degenerating cardiomyocyte present (Figure 4 C and D). Cactus group is similar to normal control (Figure 4E). In the normal control showed normal distribution of collagen (Figure 5 A). Diabetic group showed a lot of deposition of collagen (Figure 5 B). Cactus and metformin treated groups showed a little deposition of collagen (Figure 5 C and D). Cactus only group was similar in histoarchitecture with the normal control group (Figure 5 2 E).

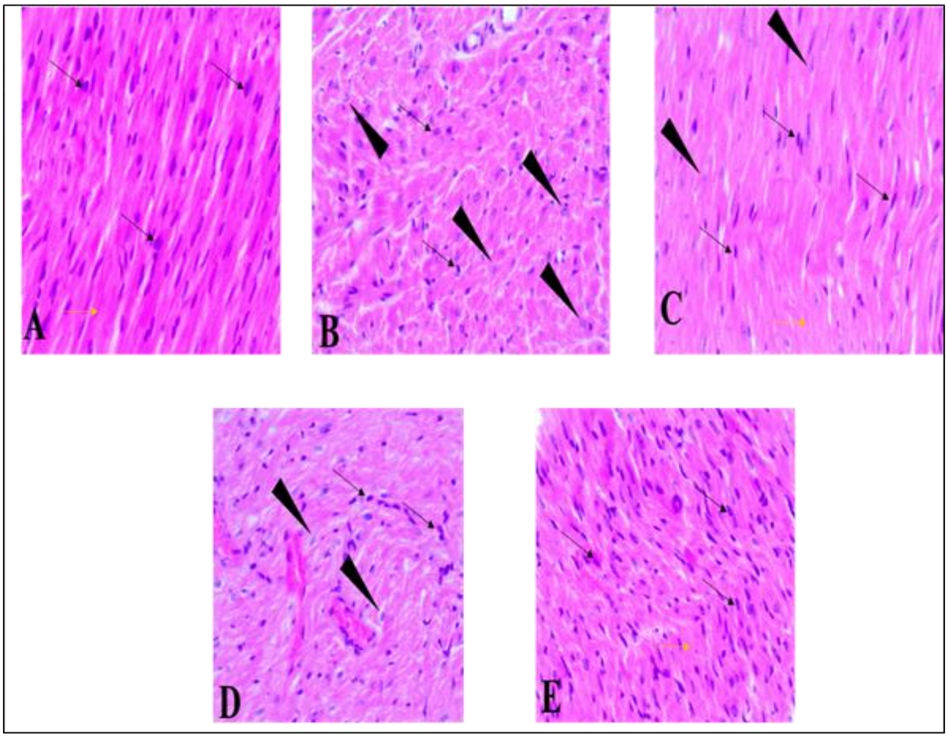

Figure 4 Photomicrograph showing the heart at day 28. H&E stain X400. A- Normal control, B – Diabetic, C – Diabetic+Cactus, D – Diabetic+Metformin and E- Cactus only. Black arrow – cardiomyocyte, Arrow head – degenerating cardiomyocyte, yellow arrow – myocardial fibers

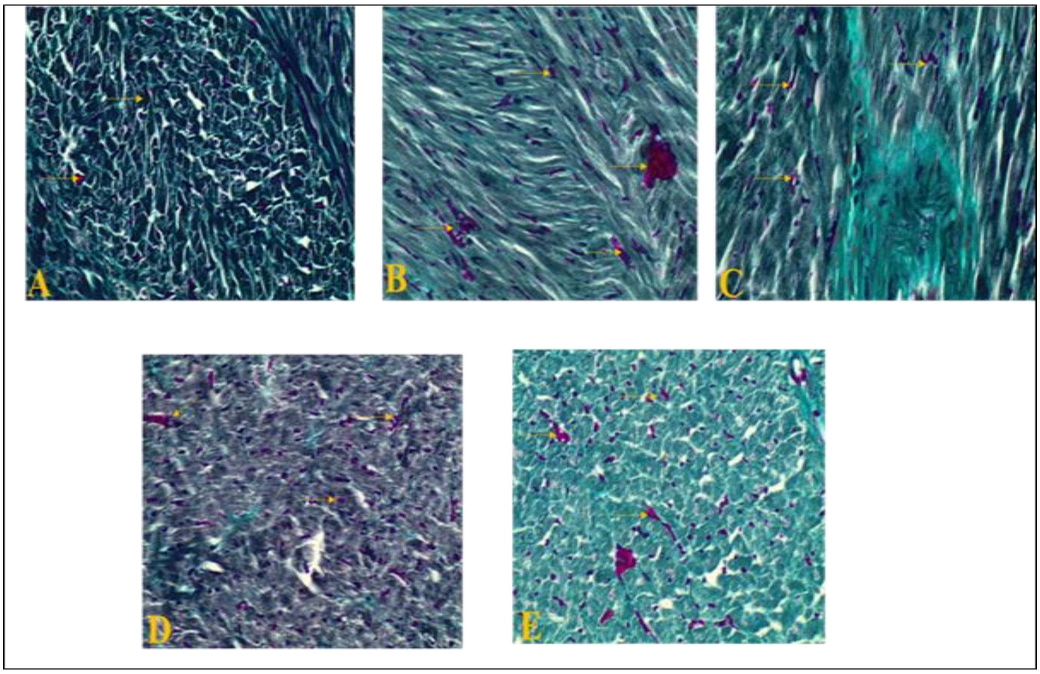

Figure 5 Photomicrograph showing the heart at day 28. MT stain X400. A- Normal control, B – Diabetic, C – Diabetic+Cactus, D – Diabetic+Metformin and E- Cactus only. yellow arrow – Collagen

4. Discussion

Cactus is reported to have 'anti-hyperglycemic and anti-diabetic (18) properties in humans and it is prescribed in many traditional and complementary therapies around the world for managements of diabetes mellitus. This present study explains the possible ameliorative effects of aqueous extract of Cactus on the diabetic heart of Wistar rat.

The results of present study showed that the diabetic Wistar rats started losing their body weight in week three due to low production of insulin by the pancreas which lead to sourcing of energy from the adipose tissue in the body due to inability to convert or breakdown the glycogen to glucose leading to rapid body weight loss in Wistar rats, causing them to source energy from adipose tissue which leads to gluconeogenesis which is in agreement with (19) the diabetes + cactus group had an increase in body weight due to chemical constituents namely antioxidants like flavonoids compounds, Quercetin, Kaempferol and isorhamnetin which were able to prevent further destruction of beta cells in the pancreatic islets as earlier reported by (20) which is capable of reducing the blood glucose levels as seen in this study. The weight of Metformin group also increased in body weight but not as compared to the cactus treated group due to suppressing gluconeogenesis, decreasing glucose output, elevating glucose uptake and utilization in peripheral tissues, and enhancing the energy metabolism in several organs, such as muscle, fat, and liver through activating of Adenosine Monophosphate activated protein kinase (21).

The relative organ weight of heart in diabetes group was significant when compared to control group due apoptosis of the cells seen histologically in this present study and it has been reported by (22) that hyperglycemia could induce apoptosis in addition to oxidative stress by inducing the emergence of reactive oxygen species (ROS) and imbalance between oxidant and antioxidant species. In diabetes + cactus the relative organ is not significant to control.

This study elucidate the diabetic induced Wistar rat demonstrated a marked hyperglycemic state compared to the control and cactus only group Wistar rats. The administration of aqueous extract of cactus demonstrated a significant reduction in the blood glucose levels as exemplified in figure 2. The group treated with metformin demonstrated also a significant reduction in blood glucose level compared to the diabetic group, but with a minimal effect compared to other groups. The antihyperglycemic effects of cactus occurred as a result of release of insulin from B cells of islets of Langerhans of pancreas, prevention of uptake of glucose from gastrointestinal tract as seen in enzymes alpha-gluconidase or Opuntia pancreatic amylase enzyme inhibitors. These enzymes inhibit 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 cactus as reported by (16).

The present study histological investigation evaluated normal histoarchitecture with numerous healthy cardiomyocyte of the heart in the normal control group (Plate:1A) as well as is cactus group (Plate 1 E),this was due to ability of cactus to reduce absorption of water-soluble dietary fiber content by interrupting absorption of glucose levels in intestines ,suppression of reactive oxygen species accumulation (antioxidant), anti-inflammatory, and autophagy-regulating effects which is in agreement with (23). Diabetic group showed that the histoarchitecture was disrupted with many degenerating cardiomyocyte (Figure 4 B) due to that in diabetes increased levels of glucose residues and metabolites upregulate the production of advanced glycation end products (AGEs), which can affect cardiomyocyte and endothelial cells. Further, during the early stages of diabetes, a lack of insulin or insulin resistance induces a metabolic shift in cardiomyocyte, whereby fatty acid intake and β-oxidation are increased to maintain sufficient levels of Adenosine triphosphate (ATP) production. However, over time, β-oxidation cannot adequately metabolize all incoming fatty acids, resulting in intracellular lipid accumulation and lipotoxicity.

Increased intracellular fatty acid concentration and mitochondrial dysfunction lead to increased generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which together increase oxidative stress and endoplasmic reticulum (ER) stress and inhibit autophagy.

Together, these effects contribute to cardiomyocyte death, cardiac hypertrophy and inflammation and a progressive profibrotic response that induces extracellular matrix (ECM) remodeling and fibrosis .Furthermore, disrupted Ca^{2+} cycling and increased fibrotic scarring in the diabetic heart can mediate contractile dysfunction and arrhythmia, contributing to heart failure and death this is in agreement with this study (23) .

Cactus and Metformin treated groups showed little disruption in their histoarchitecture and there are both healthy and degenerating cardiomyocyte present (Figure 4 C and D) this was due to increased phosphorylation of activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS), and reduced the expression of tumor necrosis factor (TGF-β1), basic fibroblast growth factor (bFGF), and tumor necrosis factor (TNF)-α (24).

Additionally, the normal control showed normal distribution of collagen (Figure 5 A) which was similar in histoarchitecture with the cactus only group (Figure 5 E) due to normal balance between synthesis and degradation of collagen in heart which is in agreement with this study (25). Diabetic group showed a lot of deposition of collagen (Figure 5 B) due to the cardiac fibroblast response to diabetes condition to produce excess extracellular matrix (collagen)which aligns with fibrosis seen in the diabetic heart (26). Cactus and Metformin treated groups showed a little deposition of collagen (Figure 5 C and D) that resulted in the diastolic stiffness through advanced glycosylation which is in agreement with this study (27).

5. Conclusion

The antihyperglycemic effect of Aqueous extract of cactus was elicited and it was seen that cactus was of great importance in the management of hyperglycemia in diabetes mellitus as it can improve glucose tolerance by decreasing postprandial hyperglycemia and also ameliorate the destruction caused on the histoarchitecture of the heart in streptozotocin induced diabetes Wistar rats based on the findings of this present research study.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There was no conflict of interest.

Statement of ethical approval

The ethical approval and permission for the study was obtained from Mulungushi University School of Medicine and Health Sciences Research ethics committee.

References

[1] Li W,Huang E ,Gao S. Type1 Diabetes mellitus and Cognitive Impairments: A Systemic Review.J Alzheimers Dis. 2017, 57(1):29-36.

[2] Harreiter J, Roden M. Diabetes mellitus – Definition, classifikation, Diagnose, Screening und Prevention (U [Diabetes mellitus-Definition, classification, diagnosis, screening and prevention. Review German. 2019, 131(1):6-15

[3] Petersmann A, Muller – Wieland D, MullerUA, LandgrafR ,NauckM, FreckmannG, HeinemannL, Schleicher E.(2019). Definition ,Classification and Diagnosis of Diabetes Mellitus. Exp Clin Endocrinol Diabetes. 127(S 01): S1-S7.

[4] Kerner W, Bruckel J, German Diabetes Association. Diabetes Association .Defination,classification and diagnosis of diabetes mellitus. Exp Clin Endocrinol Diabetes. 2014, 122(7):384-6.

[5] Sicree Richard, Jonathan Shaw, Type 2 diabetes: An epidemic or not, and why it is happening,Diabetes & Metabolic Syndrome. Clinical Research & Reviews. 2007, 1(2): 75-8.

[6] Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, Nat Rev Endocrinol. 2018, 14(2):88-98.

[7] Edwards JL, Vincent AM, Cheng HT. Diabetic neuropathy: mechanisms to management .Pharmacology and therapeutics. 2008, 120(1):1-32.

[8] Uthman A Yusuf, Olusola A. Adeeyo, Emmanuel O. Salawu, Bernard U Enaibe, Olusegun D. Omotoso. Allium cepa Protects Renal function in Diabetic Rabbit. World J. life Sci. and Medical Research. 2012; 2 (2): 86-90.

[9] El-Mostafa K, El Kharrassi Y, Badreddine A, Andreoletti P, Vamecq J, El Kebbaj MS, Latruffe N, Lizard G, Nasser B, Cherkaoui-Malki M.Nopal cactus (Opuntiaficus-indica) as a source of bioactive compounds for nutrition, health and disease. Molecules. 2014, 19(9):14879-901.

[10] Edvan, R.L., Mota, R.R.M., Dias-Silva, T.P. et al.Resilience of cactus pear genotypes in a tropical semi-arid region subject to climatic cultivation restriction. Sci Rep. 2020, 10, 10040 . https://doi.org/10.1038/s41598-020-66972-0

[11] Saad Anouar Ben, Brahmi Dalel, Ilhem Rjeibi,Amani Smida, ,Sana Ncib, d Nacim Zouari, and Lazhar Zourgui , Phytochemical, antioxidant and protective effect of cactus cladodes extract against lithium-induced liver injury in rats. 2017; 55(1): 516–525.

[12] Uthman Ademola Yusuf, Francine Kafula, Kalande Kaimba, Wandi Kalipenta, Hellen Kabwe, John Amos Mulemena, Kingsley M Kamvuma, Sam Beza Phiri, Michelo Miyoba, Bolaji Samuel Mesole, Adrian Phiri, Bwalya Bupe Bwalya, Wilson Zimba. The Role of an Aqueous Extract of Cactus on Histopancreatic Architecture in Streptozotocin Induced Diabetes Mellitus in Wistar Rats. European Journal of Pharmaceutical Research, 2022,2(2):11-16

[13] Kalungia Chichonyi Aubrey, Mary Mataka, Patrick Kaonga, Angela Gono Bwalya, Lavina Prashar, Derick Munkombwe Opuntia stricta Cladode Extract Reduces Blood Glucose Levels in Alloxan-induced Diabetic Mice. J. diabetes. 2018, 7(1): 1-11

[14] Uthman Ademola Yusuf , Hellen Kabwe , Francine Kafula , kalande Kaimba , Wandi Kalipenta, John Mulemena, Sam Beza Phiri, Michelo Miyoba, Adrian Phiri , Bwalya Bupe Bwalya , Isabel Namfukwe Luambia1, Precious Simushi 7and Aminat Adejoke Yusuf . Streptozotocin and diabetes: Modulatory role of an aqueous extract of cactus on kidney histo-architecture of model. GSC Advanced Research and Reviews, 2022, 12(02), 164–172.

[15] Yusuf Uthman Ademola, Kalipenta Wandi, Kalande Kaimba, Francine Kafula, Kalipenta, Kabwe Hellen, Warren Chanda, Michelo Miyoba, Sam Bezza Phiri, Christopher Newton Phiri, Aminat Adejoke Yusuf and John Amos Mulemena. Cactus Extract and Anti-diabetic: hepatoprotective effect in Diabetic Wistar Rats. WJPPS. 2022. 11(7):16-27

[16] Yusuf U. Ademola., Sijumbila Gibson., Mesole B. Samuel., Mulemena J. Amos.,Kamvuma Kingsley., Eweoya O. Olugbenga., Adenowo K. Thomas. Histological study on the effects of aqueous extracts of citron leaf on pancreas of hyperglycemic wistar rats. Issue of Biological sciences and pharmaceutical research. 2019; 7(5);82-90

[17] Yusuf Uthman Ademola , Kalande Kaimba, Francine Kafula, Kalipenta, Kabwe Hellen, Kalipenta Wandi, Mesole B Samuel, Kingsley Kamvuma, Warren Chanda, Michelo Miyoba, and Sam Bezza Phiri. Some of the effects of

Aqueous extract of Cactus on the Histology of Cerebellar Cortex of Streptozotocin induced Diabetic Wistar Rats. ACTA Scientific Anatomy. 2022. 1(5):2-9

[18] Mohamed Bouhrim , Noureddine Bencheikh , Hamada Imtara ,Nour Elhouda Daoudi , Hamza Mechchate , Hayat Ouassou , Loubna Kharchoufa, Mostafa Elachouri , Hassane Mekhfi , Abderrahim Ziyyat , Abdelkhaleq Legssyer , Mohammed Aziz , and Mohamed Bnouham Protective Effect of Opuntia dillenii (Ker Gawl.) Haw. Seed Oil on Gentamicin-Induced Nephrotoxicity: A Biochemical and Histological Analysis. Scientific World Journal. 2021, ID 2173012 | https://doi.org/10.1155/2021/2173012

[19] Shao-jie, Pang , ,Man, Qing-Qing ,Song, Shuang, Song, Peng-Kun , Liu, Zhen ,Li, Yu-Qian , AJia, Shan-Shan,Wang, Jing-Zhong, Zhao, Wen-Hua, Zhang, Jian, Relationships of Insulin Action to Age, Gender, Body Mass Index, and Waist Circumference Present Diversely in Different Glycemic Statuses among Chinese Population. J. Diabetes Res. 2018 doi - 10.1155/2018/1682959.

[20] Silva CFM, Saunders C, Peres W, Folino B, Kamel T, Dos Santos MS, Padilha P. Effect of ultra-processed foods consumption on glycemic control and gestational weight gain in pregnant with pregestational diabetes mellitus using carbohydrate counting. Peer J. 2021, 1;9:e10514

[21] Nan Hu, Qi Zhang, Hui Wang, Xuping Yang, Yan Jiang, Rong Chen, and Liying Wang1 Comparative Evaluation of the Effect of Metformin and Insulin on Gut Microbiota and Metabolome Profiles of Type 2 Diabetic Rats Induced by the Combination of Streptozotocin and High-Fat Diet. Front Pharmacol. 2022, 12:794103.

[22] Arnaud Tauffenberger and Pierre J. Magistretti Reactive Oxygen Species: Beyond Their Reactive Behavior Neurochem Res. 2021, 46(1): 77–87.

[23] Tang Cheng , Kai Zhou, Yichen Zhu, Wendi Zhang, Yong Xie, Zhaoming Wang, Hui Zhou, Tingting Yang, Qiang Zhang, Baocai Xu,Collagen and its derivatives: From structure and properties to their applications in food industry. Food Hydrocolloids. 2022, 131: 107748.

[24] WangYing, Sangeun Park, NeilS. Bajpayee, Yoshiko Nagaoka, Guylain Boulay, Lutz Birnbaumer. Augmented glucose-induced insulin release in mice lacking Go2, but not Go1 orGi proteins. Proc Natl Acad Sci 2011, 108(4):1693-8.

[25] Kristen L and Zhijie W. Extracellular Matrix in Cardiac Tissue Mechanics and Physiology: Role of Collagen Accumulation. Intechopen. 2021 DOI 10 .5772/intechopen .96585

[26] Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010, 10;376(9735):124-36.

[27] Rajan R, Li, WCV Wong, DG Reid, MJ Duer, VJ Somovilla, N Martinez-Saez, GJL Bernardes, R Hayward, CM Shanahan In situ characterization of advanced glycation end products (AGEs) in collagen and model extracellular matrix by solid state NMR. Chem Commun. 2017, 53(100): 13275-13380