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Research Paper

Neuroprotective Effect of Aquaous Extract of Cactus on the Hippocampus of the Streptozotocin Induced Diabetic Wistar Rats

Ally Siabwacha[1], Isabel Namfukwe Luambia[2], Jamia Milanzi[2], Sharon kaundu[3], Mwitwa kombe[3], Lukundo Mulambia Siame[1], Uthman Ademola Yusuf[1*]

[1]Department of Human Anatomy, School of Medicine and Health Sciences, Mulungushi University, Livingstone Campus, Zambia

[2]School of Nursing and Midwifery, Mulungushi University, Kabwe, Zambia

[3]Department of Clinical sciences, School of Medicine, Eden University, Lusaka, Zambia

Corresponding Author: * Uthman Ademola Yusuf

Abstract

The Cactus have been used in traditional medicine for years by the ancient people to cure several diseases such as Diabetes mellitus, The aim of this study is to carry out investigation on the neuroprotective effects of cactus extract on hippocampus of streptozotocin induced diabetic Wistar rats by assessing the histoarchitecture and histochemical indices.

Thirty male adult Wistar rats were randomly choosed into five groups made up of six Wistar rats each, control, Cactus only, diabetic+cactus, diabetic+ metformin, diabetic only groups. The induction of Diabetes was done using Streptozotocin at a dose of 70 mg/kg bw intraperitoneally, after 72 hours of established hyperglyemia the treatment commences with aqueous extract of cactus at 100mg/kg bw and Metformin at 100 mg/kg bw respectively and was given orally for 4 weeks by using oral gastric cannula.

The results showed significant decrease in average body weight and relative brain weight in diabetic group compared to diabetic+cactus and diabetic+metformin group($p<0.05$). Diabetic group had significantly different blood glucose level from normal control, diabetic+cactus and diabetic+metformin ($p<0.05$). The histological findings showed that majority of the cells in diabetic group were necrotic while Diabetic + cactus and Diabetic + Metformin are similar to control. The analysis of the Glucose-6-phosphate dehydrogenase (G-6-PDH), lactate dehydrogenase (LDH) and reduced glutathione (GSH) activities in the Hippocampus revealed that the rats in the diabetic+cactus and diabetic+metformin are similar to the control group. The aqueous extract of cactus could be beneficial in averting the damage caused by Diabetes mellitus.

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KEYWORDS: Cactus, Hippocampus, Diabetes mellitus, streptozotocin, Hematoxylin and eosin stain.

1. INTRODUCTION

Diabetes mellitus is a chronic disease that occurs either when the pancreatic beta cells do not produce enough insulin or when the body cannot effectively use the insulin it produces.[1] It can be broadly classified into type 1 and type 2 where type 1 diabetes is as the results of autoimmune destruction of the pancreatic beta-cells and type 2 is a condition in which the insulin produced does not effectively used to maintain the blood sugar level in the body [2], and further subclassified into Gestational diabetes mellitus (GDM) which is defined as glucose intolerance that is first detected during pregnancy and the disorder has its beginning in the third trimester of pregnancy. Monogenic is another subtype type of diabetes which is characterized by impaired secretion of insulin from pancreatic β cells caused by a single gene mutation [3]. The complication of diabetes mellitus can divide into two main types the microvascular that effect on small vascular in each of retinal, peripheral nerve and kidney that lead to retinopathy, neuropathy, nephropathy, Peripheral Artery Disease, Coronary Artery Disease, Cerebrovascular disease and macrovascular complication that effected on large vascular including Peripheral Artery Disease, Coronary Artery Disease and Cerebrovascular disease [2]. Diabetes is approximately estimated to contribute to one in nine deaths among adults aged 20-79 years. Prevention of diabetes mellitus and its complications is essential, particularly in middle-income countries, where the current impact is estimated to be the largest [4]. Hippocampus plays a pivotal role in memory formation, emotional, adaptive, and reproductive behaviors and also is particularly important in forming new memories and connecting emotions and senses, such as smell and sound, to memories [5]. New memory formation and consolidation process of events by hippocampus depend on the integrity of hippocampus internal circuits [5]. Hippocampus structural complexity has made it vulnerable to the many pathological conditions such as diabetes mellitus. Any factor disturbing the balance between neuronal proliferations/death may result in memory and learning impairment. Studies have demonstrated that experimental diabetes causes decreased granular cells proliferation and neuronal death (necrosis/apoptosis) in CA3 and dentate gyrus regions [5].

According to the 2019 WHO Global Report on Traditional and Complementary Medicine, the use of traditional medicine is widespread in Africa. The report indicated that 85% of the total Member States in the WHO African Region confirmed the use of traditional medicine in the treatment of various diseases, including diabetes mellitus. Cactus has traditionally been used in Africa to treat diabetes mellitus due to its availability.[6] This research aimed to investigate the neuroprotective effects of cactus extract on hippocampus of streptozotocin induced diabetic Wistar rats by assessing the histoarchitecture and histochemical indices.

2. MATERIALS AND METHODS

Plant Materials

The cactus extract was harvested from Chipata district of Eastern 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 ground and sieved to obtain a homogenous powder (500g). The extraction was done using Soxhlet extraction methods.[7]

Animals and Animal Management.

The randomly assigned thirty adults presumably healthy male Wistar rats 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 Mulungushi University, department of human anatomy, Livingstone. They were maintained on standard animal feeds (Wealth-gate pelletized feeds) and allowed access to clean water and feeds freely.[8]

Induction of Diabetes

Streptozotocin (STZ) was used to induce diabetes. Rats was weighed, and a baseline glucose level established after the overnight fasting period. The animals were injected intraperitoneally with STZ at a dose of 70mg/kg body weight and reintroduced to the normal feeding cycle. [9] It took about 72 hours for diabetes to show in the animals following the administration of STZ therefore a fasting blood sugar was collected to determine the initiation of diabetes using the tail vein puncture. An Accu-Chek glucometer (Mannheim, Germany) was used to access blood glucose levels. Animals was considered diabetic with fasting blood glucose levels above 7 mmol/l. [9]

Experimental Design

Thirty adults male Wistar rats were randomly selected into five groups of six. Control Animals in Group A were normal control and got neither STZ nor cactus extract; those in Group B were normal and received Cactus extract only; those in Group C were treated for diabetes with Cactus extract; and those in Group D were treated for diabetes with metformin and those in Group E were diabetic but received neither Cactus nor metformin.

Mode of Administration of Cactus extracts

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

Measurement of Body Weight (g)

Body weight (g) of the rats was recorded for two weeks (acclimatisation 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);[19]. [11]

Measurement of Blood Glucose Level

The blood glucose was evaluated in overnight fasted rats at 9:00–10:00 hours using Glucose oxidase method of one touch ultra 2 glucometers (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. [11]

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 will be recorded as percentage (%) using sensitive weighing balance (SonyF3G brand); [11]

Histological and Histochemical studies

At the end of this 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 prepare formal saline for 72 hours and processed for routine histological examinations stained with Haematoxylin and Eosin (H&E) to observe changes in the cellular morphology and phosphotungstic acid-haematoxylin (PTAH) was used to observe the astrocyte. The tissues for enzymes of Glucose metabolism (Glucose 6 phosphate dehydrogenase (G6PDH) and Lactate dehydrogenase (LDH)) and oxidative stress markers (Reduced glutathione (GSH)) studies were immediately placed in 0.1M of phosphate buffer solution (PH 7.4) for homogenization.

Photomicrography

Photomicrography of histological sections of the hippocampus was taken with an Olympus Microscope (New York, United State of America) coupled with camera at University Teaching Hospital (UTH) Histopathology Department, Lusaka, Zambia.

Statistical Analysis

Data was presented as mean ± standard error of the mean (mean±SEM); analyzed using one way ANOVA and all graphs was 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

Figure 1 shows that the two weeks of acclimatization (week -2 and week -1) there was no significant changes in the body weights across all the groups ($p>0.05$). At week 0 (week of induction) and week 1(first treatment week) showed no significant changes in the body weights of all the groups ($p>0.05$). From week 2 to week 4 showed that there was decrease of average body weight in the diabetic group when compared to Control, Cactus only, Diabetic+cactus and Diabetic+metformin groups it was significant ($p<0.05$). The Diabetic+cactus and Diabetic+metformin groups had relatively similar body weight when compared to the control and cactus only groups they are not significant ($p>0.05$).

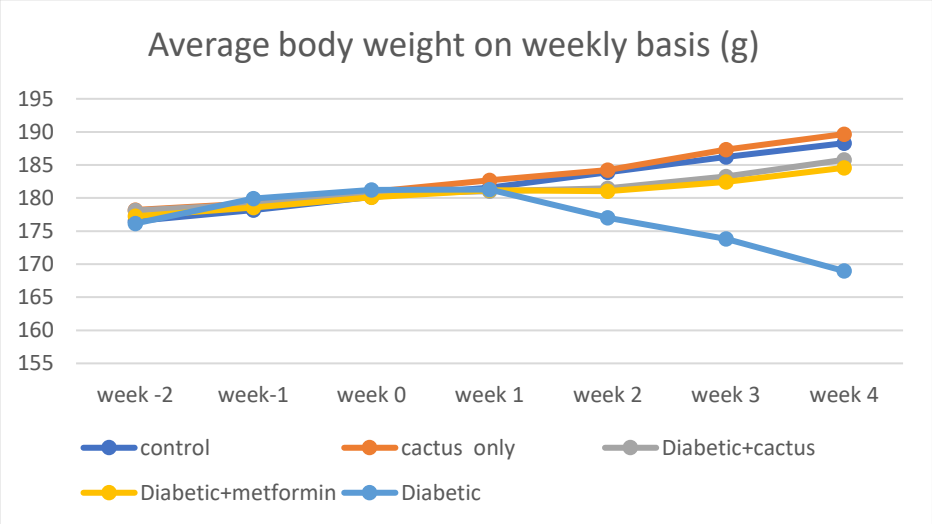

Figure 1: Effect of the Aqueous Extract of Cactus on Average body weight on weekly basis (g). Data expressed mean ± SEM $P<0.05$

Figure 2 shows that in the weeks of acclimatization (week -2, week-1), there was no significant changes of the blood glucose levels across all the groups. Week 0 (induction week) shows significant raise of the blood glucose levels in the diabetic+cactus, diabetic+metformin and diabetic groups when compared to the control and cactus only groups it was significant ($p<0.05$). There was significant reduction of the blood glucose levels in both diabetic+cactus and diabetic+metformin groups from week 1 to week 3 when compared to control and cactus only groups it was not significant ($p>0.05$). In week 4, the Diabetic cactus was similar to the control and cactus only groups when compared there was no significant difference ($p >0.05$). The Diabetic group remained high and we compared to other groups it was significant ($p<0.05$).

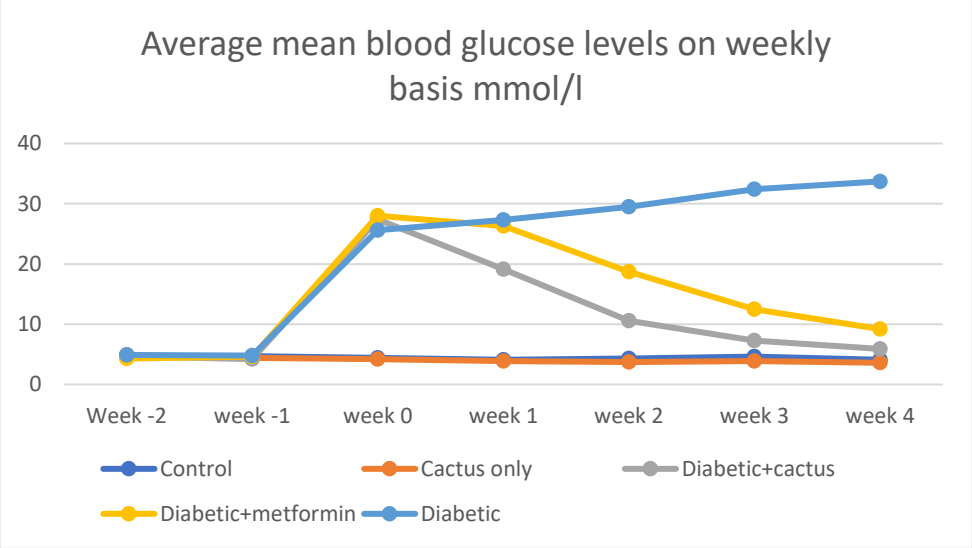

Figure 2: Effect of the Aqueous Extract of Cactus on the Blood Glucose Levels on Weekly Basis (mmol/l). Data Expressed as MEAN±SEM P<0.05

Figure 3 shows that there were no significant changes in relative brain weight of both control and cactus only groups, however, the cactus only group had slightly higher relative brain weight than the control group (p>0.05). There was significant decrease in relative brain weight of the diabetic group when compared to Diabetic+cactus and Diabetic+metformin groups (p<0.05) it was also significant when compared to the control and cactus only groups (p<0.05). There were no significant changes when the Diabetic+cactus and Diabetic+metformin groups was compared to the control and cactus only groups (p>0.05).

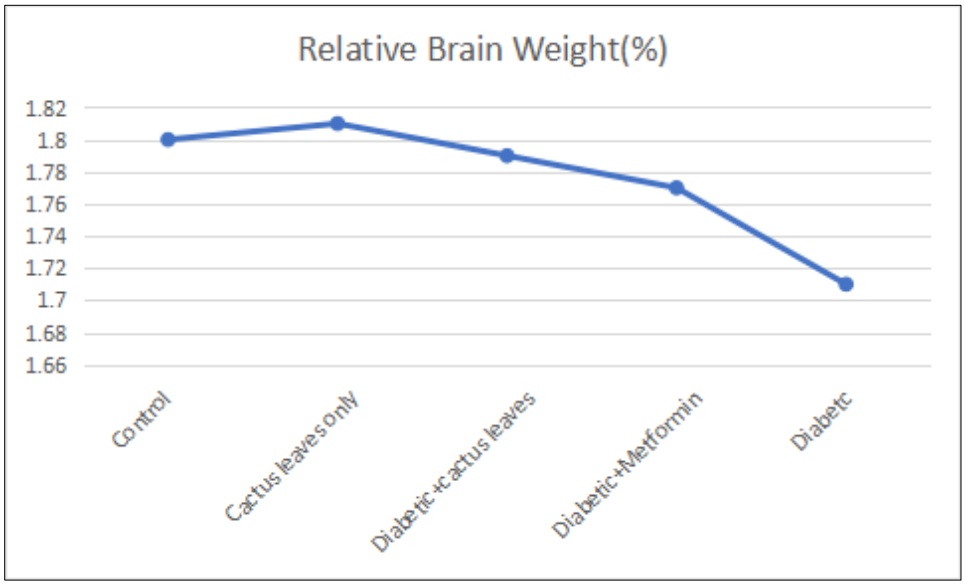

Figure 3: Effect of the Aqueous Extract of Guava Leaves on the Relative Brain Weight (%). Data Expressed as MEAN±SEM P<0.05

Histological Findings

Haematoxylin and Eosin (H&E) Stain

The rats in the control and Cactus only groups show normal histoarchitecture with densely healthy populated pyramidal neuron (Fig. 4: A and B). Diabetic+cactus group appeared similar to control (Fig. 4 C) while diabetic+metformin group, shows that some cells were necrotic while some cells appeared normal (Fig. 4 D). the rats in the diabetic group most of the cells present were necrotic and the normal cells present were very few (Fig. 4 E).

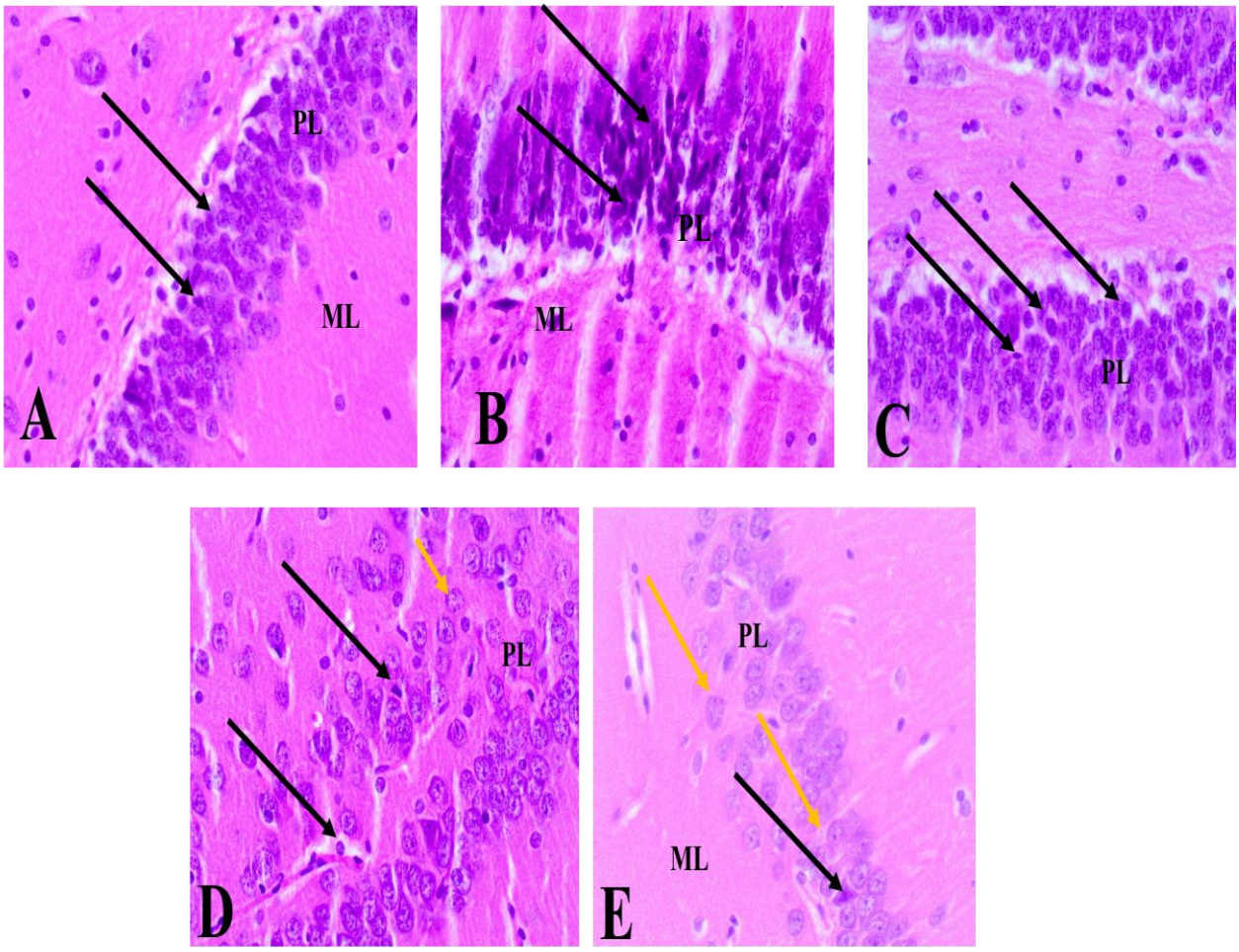

Figure 4: Photomicrograph showing the Hippocampus at week 4. H&E stain X400.

A-Normal control, **B**- cactus only, **C**- Diabetic+cactus, **D**- Diabetic+metformin, **E**-Diabetic. **Black arrow**- pyramidal cells, **yellow arrow**- Necrotic cells, **PL**- pyramidal layer, **ML**-Molecular layer.

Phosphotungstic acid-haematoxylin (PTAH) Stain

In the control and cactus only, groups showed normal distribution of astrocyte, neurons and the histoarchitecture appeared normal (Fig. 5: A and B). diabetic+cactus and diabetic+metformin groups, showed normal neurons and astrocytes but sparsely stained (Fig. 5 C and D). In the diabetic group, there were astrocytes present with degenerated neurons (Plate: 5 E).

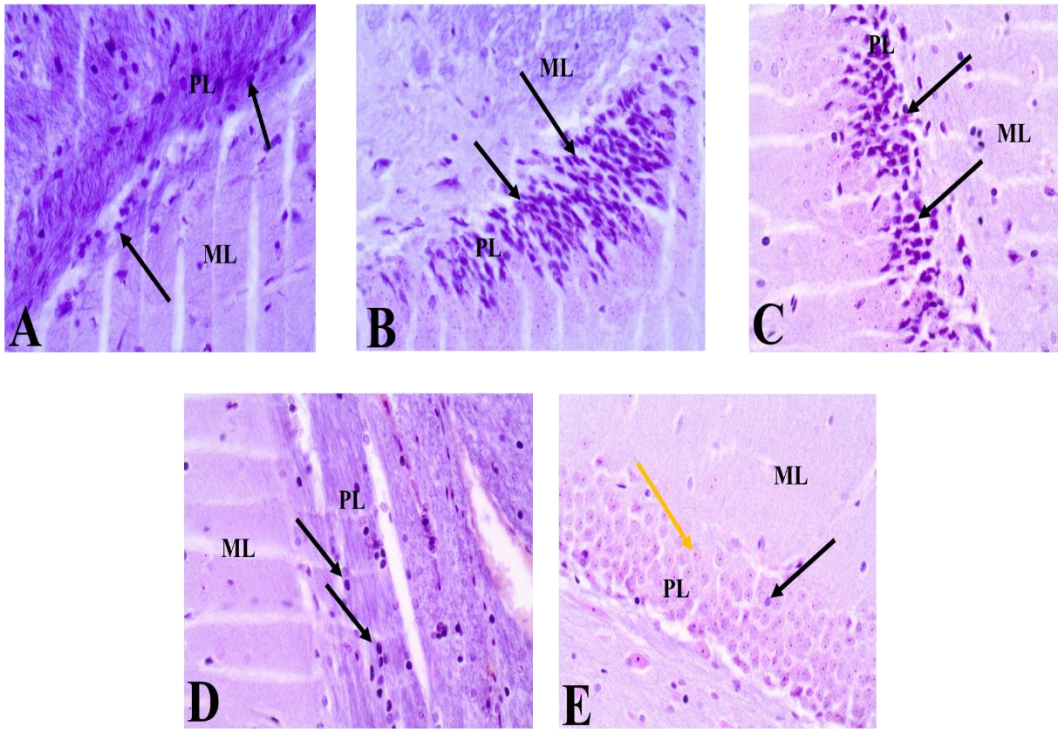

Figure 5: Photomicrograph showing the Hippocampus at week4. PTAH stain X400.

A – Normal control, **B** - cactus only, **C**- Diabetic+cactus, **D** – Diabetic+metformin,
E- Diabetic. **Black arrow** - Astrocytes, **Yellow arrow** – necrotic cells, **PL** – Pyramidal layer, **ML** – Molecular layer.

Histochemical Studies

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity in the Hippocampus (Iu/L)

Figure 6 shows that the diabetic group of rats had lowest glucose -6-phosphate dehydrogenase (G-6-PDH) activity among all the groups when compared to control, cactus only,Diabetic+cactus and diabetic+metformin groups it was statistically significant ($p<0.05$). The rats in the Diabetic+cactus group had slightly higher G-6-PDH activity than diabetic+metformin group when compared it was not significant ($p>0.05$). The normal control and the cactus only groups had similar glucose-6-phosphste dehydrogenase activity, however when compared there was no significant difference ($p>0.05$).

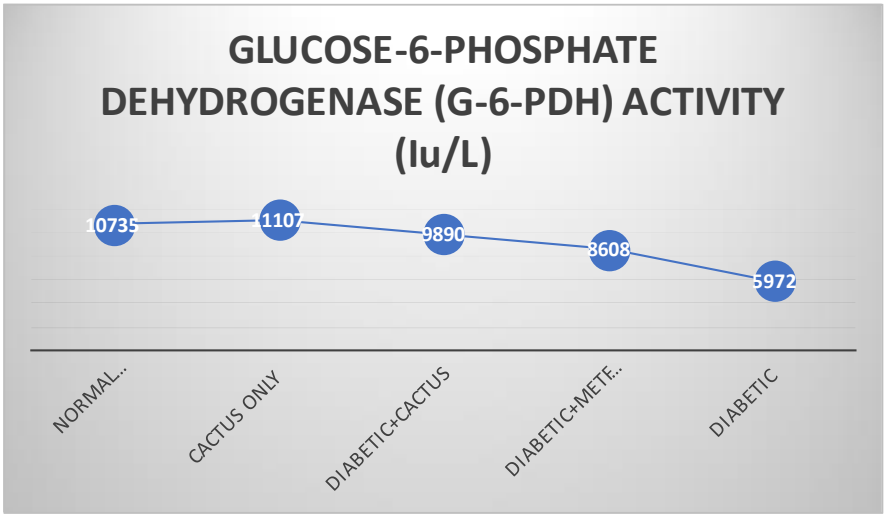

Figure 6: Glucose -6-phosphate dehydrogenase(G-6-PDH) activity level in Hippocampus (Iu/L) at the end of week 4. Data were expressed as Mean ± SEM. P<0.05

Lactate dehydrogenase (LDH) activity in Hippocampus (iu/L)

Figure 7 shows that the diabetic group had highest lactase dehydrogenase (LDH) activity among all the groups when compared to other groups it was statistically significant ($p<0.05$). The normal control and the cactus only groups had similar lactase dehydrogenase activity but the cactus only group had lowest LDH activity and when compared to each other there was no significant difference ($p>0.05$). The lactase dehydrogenase activity in diabetic+cactus and diabetic+metformin groups are similar but when compared to normal control and cactus only group, there is no significant difference ($p>0.05$).

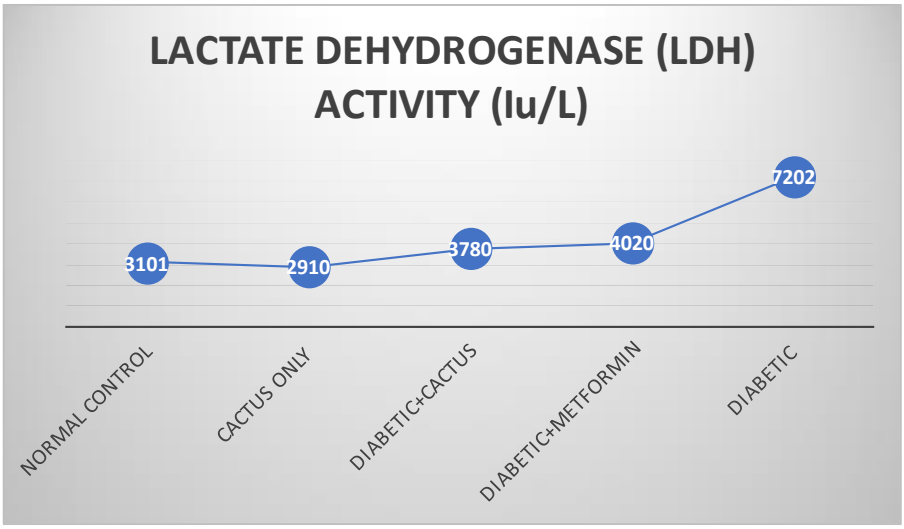

Figure 7: Lactate dehydrogenase (LDH) activity in the Hippocampus (iu/L) at the end of week 4. Data Expressed as MEAN±SEM $P<0.05$.

Reduced glutathione (GSH) activity in the Hippocampus (Iu/L)

Figure 8 shows that the normal control and diabetic+cactus groups had similar Reduced glutathione (GSH) activity when compared, there was no significant difference ($p>0.05$). Diabetic group of rats had statistically significant reduced glutathione when compared to normal control and diabetic+cactus, diabetic+metformin and cactus only groups ($p<0.05$). Diabetic+metformin and diabetic+cactus had similar GSH activity when compared to each other, there was no significant difference ($p> 0.05$).

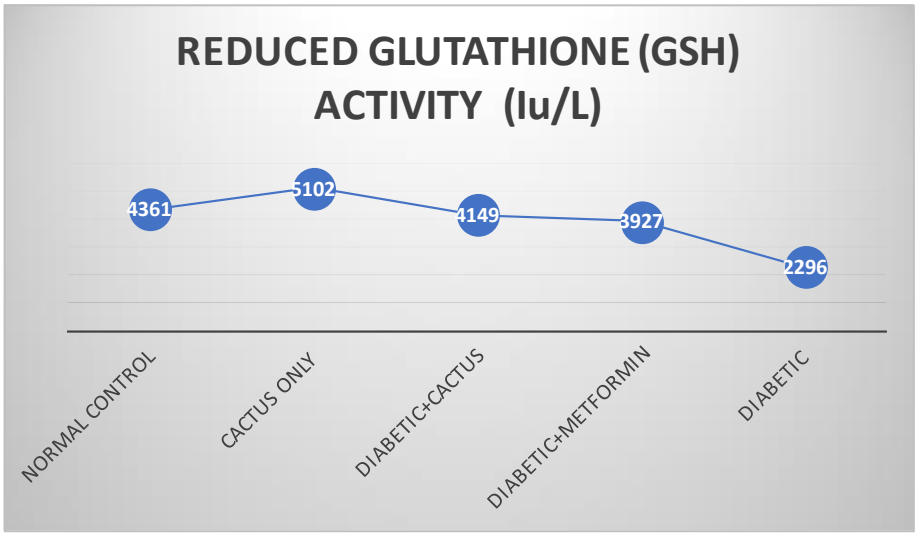

Figure 8: Reduced glutathione (GSH) activity (lu/L) level in Hippocampus at the end of week 4. Data were expressed at Mean ± SEM.$P<0.05$.

4. DISCUSSION

Cactus compounds and derivatives were shown to be endowed with biologically relevant activities including anti-inflammatory, antioxidant, antihyperglycemic, anti-microbial and neuroprotective properties [12]. This study aimed to investigate the neuroprotective effects of cactus extract on hippocampus of streptozotocin induced diabetic Wistar rats by assessing the histoarchitecture and histochemical indices.

In this current study the average body weight of the diabetic group decreased significantly compared to control group as shown in figure 1. This could be linked to increased tissue catabolism compared to anabolism which resulted in decline in tissue proteins, fats and enhanced muscle wasting due to excessive increased metabolic processes such as glycogenolysis, lipolysis and proteolysis. [13] Diabetic+cactus have had much improved average body weight gain compared to diabetic+metformin group which could be because cactus contains dietary cellulose fibre and rich source antioxidants such as vitamin C, phenolics, flavonoids compared to metformin whose antihiperglycemic agent acts primarily by decreasing endogenous hepatic output of glucose by inhibition of gluconeogenesis. This findings are in harmony with other similar studies by (9)

In this present study the average blood glucose levels of control and cactus only groups were normoglycaemic as seen in figure 2, this shows that cactus does not have hypoglycemic effects on the Wistar rats. After the week of induction (week 0), there was significant increase in blood glucose levels in the diabetic+cactus, diabetic+metformin and diabetic groups compared to control and cactus only groups, this could be caused streptozotocin(STZ) which induces DNA strand breaks and DNA alkylation that leads to necrosis of pancreatic beta cells. [14] However, the blood glucose levels in diabetic+cactus and diabetic+metformin significantly decreased until normoglycaemic states was attained at week 4 of treatment. This could be due to bioactive compounds such as phenols and flavonoids and the in vitro inhibition effects against alpha amylase and alpha glucosidase which was evaluated, these enzymes inhibit the digestion of glucose into absorbable product that would block the glucose uptake into the blood. [15] The constant rise in the blood glucose levels in diabetic group compared to the control and cactus only groups, could be attributed to specific necrosis of pancreatic beta cells caused by STZ and resulted into hyperglycemia (9).

In figure 3 the diabetic group had the lowest relative brain weight compared to other groups, this could be attributed to apoptotic markers such as Bax expression which dramatically raised at both the mRNA and protein levels, whereas Bcl-xL and Bcl 2 expression was significantly reduced, implying that hyperglycemia-induced apoptosis in hippocampus of diabetic rats could be mediated by mitochondria. [13] It could also be related to the fact that cholesterol in the body come from in situ synthesis therefore in an insulin-deficient diabetic rats, diabetes causes reduction in expression of the major transcriptional regulator of cholesterol metabolism, SREBP-2 and its downstream genes in the hypothalamus and other areas of the brain leading to a reduction in brain cholesterol synthesis and synaptosomal cholesterol content [12]. There was improvement in relative brain weights in diabetic+cactus and diabetic+metformin groups which was nearly the same as control and cactus only groups. Diabetic+cactus group had more improved relative brain weight than diabetic+metformin group. This could be attributed to cactus extracts that have shown to have neuroprotective effects and reduce oxidative stress in the brain because active constituents such as indicaxanthin, a bioavailable betalain pigment which has demonstrated to cross blood-brain barrier (BBB) and modulate redox-dependent signaling pathways, exerting significant anti-oxidative and ant-inflammatory effects in diabetic+cactus rats. [15]

The histological findings by use of Hematoxylin and Eosin (H& E) stain showed that figure 4 E photomicrograph had the most necrotic cells in the pyramidal layer of the hippocampus signifying the complication of the diabetes in this group of rats. This is in line with the report by [16] who reported that the considerable rise in apoptotic markers including Bcl-2, Bcl-xl, Bax and caspases 3 was observed in the hippocampus environment of the diabetic mice in several preclinical studies. Also, few studies suggest that blood brain barrier (BBB) permeability is reduced in diabetic animals' models due to degeneration of the cerebral vasculature.[13] The photomicrograph in figure 4 D showed normal cells and some trace of necrotic cells suggesting that hyperglycemia affected some cells before being controlled. Photomicrograph in figure 4C was similar to photomicrographs figure 4 B and figure 4 A which presented with normal histoarchitecture of hippocampus. This could be attributed to cactus extract having both decreased the intestinal absorption of glucose and the chemical component indicaxanthin that cross BBB barrier and accumulate in different areas of the brain and exerting the neuroprotective effects [13]. Figure 4 A and figure 4 B had normal histoarchitecture. It shows that cactus did not have toxic effect on the cactus only group (figure 4 B).

The hippocampus photomicrograph figure 5 E of the diabetic rats using phosphotungstic acid- haematoxylin (PTAH) staining at the end of week 4 showed necrotic cells and increased pale pigmented number of deformed astrocytes in the pyramidal layer compared to normal control (figure 5 A) and cactus only (figure 5 B). This could be attributed to hyperglycemia-induced neurotoxicity which is mainly due to increased production of advanced glycation end products (AGEs), increased polyols pathway flux, activation of proteins kinase C(PKC) isoforms and increased hexosamine pathway influx, all of which leads to an increased in oxidative damages and vascular complication (5).Also hyperglycemia leads to the formation of reactive oxygen species (ROS), other oxidative stress markers and reactive nitrogen species(RNS) [17]. Astrocytes can phagocytose synapses, alter neurotrophin secretion and clear debris and increased number of astrocytes in the brain signifies an active inflammatory state. [13] The photomicrographs figure 5 C and figure 5 D showed similar cytoarchitecture of astrocytes cells and no trace of abnormal cells. However, photomicrograph (figure 5 C) of diabetic+cactus showed normal cytoarchitecture

of astrocyte to near normal control (figure 5 A) and cactus only (figure 5 B) compared to diabetic+metformin (figure 5 D).This could be due to the active components of cactus as eluded by [13] that cactus extract decreases the intestinal absorption of glucose and have chemical component indicaxanthin that cross BBB barrier, accumulate in different areas of the brain and exerting the neuroprotective effects. Photomicrographs figure 5 A and figure 5 B had normal cytoarchitectures of pyramidal, molecular layers and astrocytes. This shows that cactus had no neurotoxic effects on the cactus only group of Wistar rats.

The histochemical study of glucose-6-phosphate dehydrogenase (G-6-PDH) activity (lu/L) showed statistically significant decline in the diabetic group compared to the normal control and cactus only groups of rats. This is because chronic hyperglycaemia caused inhibition of G-6-PDH activity via decreased expression and increased phosphorylation of G-6-PDH which therefore led to increased oxidative stress as reported by [17]. High blood glucose can cause activation of protein kinase A (PKA) and subsequent phosphorylation and inhibition of G-6-PDH activity and hence decreased nicotinamide adenine dinucleotide phosphate (NADPH) [18]. Following Figure 6 the normal control and cactus only groups had similar G-6-PDH activity, this entails that cactus extract had no inhibiting effects on G-6-PDH. Diabetic+metformin group of rats had activity of G-6-PDH which was close to that of Diabetic+cactus group. Diabetic+cactus group had notably slightly raised G-6-PDH activity, this could be attributed to bioactive compounds (phenols and flavonoids) and the in vitro inhibition effects against alpha amylase and alpha glucosidase which was evaluated. [10]

In the figure 7 the enzyme of lactate dehydrogenase (LDH) activity shows that, the diabetic group of rats was observed to have the highest LDH activity this could be linked to a report by [19] who reported that hyperglycaemia lead to an increased polyol pathway flux; increased advanced glycation end- product (AGE) formation; activation of protein kinase C (PKC) isoforms; and increased hexosamine pathway flux- ultimately generating an elevated NADH/NAD+ ratio and lactate production. Increased level of LDH reflects the adverse effect of diabetes. The lactate dehydrogenase activity levels in diabetic+cactus and diabetic+metformin were similar, however diabetic+cactus had less activity level which could be aligned to cactus having bioactive compounds such as phenols and flavonoids and in vitro inhibition effects against alpha amylase alpha glucosidase [20]. The normal control and cactus only groups had similar levels of lactate dehydrogenase activities, respectively, this signifies that cactus extract has no effect on the lactate dehydrogenase enzyme.

Glutathione reductase is responsible for maintaining the supply of the reduced glutathione (GSH); one of the most abundant reducing thiols in the majority of cells. In its reduced form, glutathione plays key roles in the cellular control of reactive oxygen species (4). The an antioxidant enzymes such as glutathione reductase determine the most suitable conditions for redox control within a cell or for activation of programmed cell death. [21] In this study, the GSH activity of normal control and diabetic+cactus is similar compared to cactus only. The cactus extract had an effect on GSH by increasing the activity. This could be related to phytochemicals present in cactus, its antioxidant benefits may be attributed to synergistic effect between betalains and flavonoids. The diabetic group had lowest GSH activity compared to other groups, this could be linked to decrease in the reduced GSH level and impairment in GSH metabolism which has been reported in the erythrocytes of diabetics [12]. Also the decreased in the level of GSH could be due to the competition between aldose reductase and glutathione reductase for NADPH, a cofactor, and increased oxidative stress (increased ratio of NADH/NAD). [22] The GSH activity of diabetic+cactus and diabetic+metformin groups are similar but diabetic+cactus was slightly higher. It might be because of the phytochemicals present in cactus and its antioxidant benefits as reported by. [7]

5. CONCLUSION

The aqueous extract of cactus possesses antihyperglycemic properties which was able to lower the blood glucose level faster than the metformin, improves body weights and relative brain weights, it complements the activities of enzymes for glucose metabolism, biomarker of oxidative stress and also improves histoarchitecture of hippocampus of the diabetic rats.

6. DECLARATIONS

Competing interests

The authors declare that they have no competing interest.

Ethics approval and consent to participate

The ethical and regulatory approval was obtained from Mulungushi University School of Medicine and Health Sciences Research Ethics committee and the National Health Research Authority, Zambia. This study was conducted at the Animal Holdings of the Mulungushi University, Department of Human Anatomy, Livingstone, Zambia. The animals were maintained on standard animal feeds (Wealth-gate pelletized feeds) and allowed access to clean water and feeds freely.

7. Consent for Publication

Not applicable, this study does not include publishing of personal data

Availability of data and materials

Data supporting our finding can be found with the corresponding author. Data will not be shared. Please contact the author for data request.

8. Funding

This research was self-funded by the authors

9. AUTHOR'S CONTRIBUTION

Isabel Namfukwe Luambia – Research design.

Ally Siabwacha - Incharge of Animal euthanasia and Dissection.

Memory Ngosa- Preparation of extract.

Sharon kaundu - Responsible for Animal holding and cares.
Mwitwa kombe - Responsible for preparing tissue homogenate and enzyme assays.
Lukundo Mulambia Siame - Responsible for Tissue processing.
Mulenga Malata – data analysis.
Uthman Ademola Yusuf - Histological slides Interpretation and ensure study is conducted in an ethical manner.

Cover letter
This manuscript is submitted to International Journal of Contemporary Research in Multidisciplinary because the information included is within the scope of the journal of medicine, neuroscience. We confirm that this manuscript is not presently under submission in any other journal and all the authors have agreed to submission to this journal.

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