Acta Scientific ANATOMY

Volume 1 Issue 5 June 2022

Research Article

The Effects of Aqueous Extracts of Cactus on the Cerebellar Cortex of Streptozotocin Induced Diabetic Wistar Rats

Uthman Ademola Yusuf[1]*, kalande Kaimba[1], Francine Kafula[1], Kalipenta Wandi[1], Kabwe Hellen[1], Kingsley M Kamvuma[2], Sam Beza Phiri[3], Michelo Miyoba[4], Bolaji Samuel Mesole[5] and Wilson Zimba[6]

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

[2]Department of Pathology and Microbiology, School of Medicine and Health Sciences, Mulungushi University, Livingstone Campus, Zambia

[3]Department of Physiological Sciences, School of Medicine and Health Sciences, Mulungushi, University, Livingstone Campus, Zambia

[4]Department of Clinical Sciences, School of Medicine and Health Sciences, Mulungushi, University, Livingstone Campus, Zambia

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

[6]Public Health Unit, School of Medicine and Health Sciences, Mulungushi University, Livingstone Campus, Zambia

***Corresponding Author:** Uthman Ademola Yusuf, Department of Human Anatomy, School of Medicine and Health Sciences, Mulungushi, University, Livingstone Campus, Zambia.

Received: June 06, 2022

Published: June 27, 2022

Abstract

Cactus (Opunta Species) has been one of the herbs used in the management of several ailment such as diabetes mellitus, cardiovascular diseases, tumour for years. Cactus has been reported to contain antihyperglycaemic effects. It is very common in rural settings of sub-saharan Africa and is now being adopted in metropolitan areas. The effects of cactus extracts on the body weight, relative organ weight, blood glucose and the histoarchitecture of cerebellar cortex of streptozotocin induced diabetic Wistar rats were investigated. Thirty male Wistar rats weighing 160g to 200g were randomly divided in five groups each comprising of six rats; namely normal control, diabetes, diabetes + cactus (100 mg/kg), diabetes + metformin (100 mg/kg) and cactus only (100 mg/kg). Cactus extracts and metformin were orally administered to the animals daily for four weeks. Cactus extracts improved the body weight and blood glucose level (normal range) in diabetic rats. The photomicrographs of the normal control and cactus only groups showed normal histoarchitecture of the cerebellar cortex. The histological findings in diabetes group showed numerous degenerating Purkinje cells whereas diabetes + cactus and diabetes + metformin exhibited less degenerating Purkinje cells. Aqueous extracts of cactus administration has been shown to increase body weight, no significant difference in the relative weight of the brain, maintains blood glucose level within the normal range and improves the histoarchitecture of the cerebellar cortex of streptozotocin induced Diabetes mellitus male Wistar rats.

Keywords: Streptozotocin; Cerebellar Cortex; Cactus Extracts; Metformin; Diabetes Mellitus

Introduction

Diabetes mellitus is a syndrome of disordered metabolism usually due to a combination of hereditary and environmental factors that results in abnormally high blood glucose levels [4]. Blood glucose levels are regulated by complex interaction of different chemicals and hormones in the body. Insulin is one of the hormones that plays a key role in the control of blood glucose levels. It is made and secreted by the beta cell of the pancreas [24].

Diabetes mellitus develops due to diminished production of insulin (in type 1) or resistance to its effects (in type 2 and gestational), [19]. In both circumstances, hyperglycaemia is the end result. At an advanced stage, diabetes mellitus affects other metabolic pathways of lipids and manifests as hypercholesterolemia and hyperlipidemia which are risk factors for atherosclerosis [5]. The progression of the disease affects various vital organs in the body.

Diabetes is accompanied by overproduction of free radicals. Hyperglycaemia induced mitochondrial reactive oxygen species production could be the key episode in the progress of diabetic complication [2]. Increased production of the reactive oxygen species and at the same time decline in antioxidants observed in diabetic patients could promote the development of the complications [10].

The complications that occur in diabetes mellitus are grouped into microvascular and macrovascular. Microvascular is attributed to damage of the small blood vessels while macrovascular involves damage to the arteries [2]. Microvascular include retinopathy, nephropathy, neuropathy and diabetic foot whilst major macrovascular complications include accelerated cardiovascular disease leading to myocardial infarction and cerebrovascular disease manifesting as stroke [1]. Other chronic complications of diabetes include depression [9] dementia and sexual dysfunction [2].

Diabetes mellitus is one of the commonest non-communicable disease worldwide. In 2019, International Diabetes Forum (IDF) reported that approximately 463 million adults (20-75 years) were living with diabetes and caused 4.2 million deaths. More than 1.1 million children and adolescents are living with type 1 diabetes whereas more than 20 million live births (1 in 6 live births) are affected by diabetes during pregnancy. 374 million people at risk to develop type 2 diabetes [13].

According to International Diabetes Forum (IDF), 19.4 million adults aged 20 to 79 years were living with diabetes in the IDF African region with highest proportion of undiagnosed diabetes, where 60% of adults currently living with diabetes are unaware of their condition [13].

Diabetes mellitus has emerged as one of the major contributors of morbidity and mortality worldwide. The conventional medicine takes charge of diabetes become cumbersome with the appearance of complications. Diet, exercise, modern drugs including insulin and oral hypoglycaemic drugs such as sulfonylurea and biguanides are used in the management of diabetes mellitus [19]. Herbal medicine have been reported as one of the remedies used for the treatment of diabetes in different countries such as China, Vietnam, India, Nigeria, Oman and Zimbabwe [12]. Different medicinal plants are used for the traditional management of diabetes in the mentioned countries and many others. Cactus has antihyperglycemic effects and can ameliorates the diabetic complication in Wistar rats. This is attributed to its phytochemicals properties. It has been used in various studies and its antihyperglycemic effects have been found to be remarkable.

Methods and Materials

Animals and animal management

Thirty adult male Wistar rats weighing between 160g and 200g were used for this study. The animals were acclimatized for one week and housed in the animal holdings of the Department of Anatomy, Mulungushi University School of Medicine and Health Sciences, Livingstone Zambia. After acclimatization, the animals were divided into five groups of six rats per group. They were maintained on standard animal feeds and allowed access to clean water and feeds freely.

Plant materials

The Cactus was harvested from Livingstone district, Southern Province of Zambia. The Cactus was air dried for six weeks and pounded. The dry pounded Cactus was then grounded and sieved to obtain a homogenous powder. The aqueous extraction was done using Saaad methods [7].

Experimental procedure

Thirty adult Wistar rats weighing between 160 and 200g were used. The animals were acclimatized for one week. They were kept in cages and were maintained on standard animal feeds (mouse pelletized feeds) and access to clean water and feed freely (*ad* libitum) throughout the experimental period.

Induction of diabetes in wistar rats

Before induction, the thirty animals were weighed, and a baseline glucose level was established by using a glucometer (Accu-

Chek Compact Plus) after the overnight fasting period. The animals were divided into five groups at random namely; normal control, diabetes, diabetes + cactus, diabetes + metformin and cactus only for which only three groups were induced with diabetes. Streptozotocin calculated at a dose of 70 mg/kg body weight and administered intraperitoneally at a single dose [3,14]. Streptozotocin was freshly prepared and kept on ice before use.

The dose of streptozotocin used for induction was calculated using the formula: dose volume = (weight single animal/Total weight of animals in the group) × 5ml solution of streptozotocin.

Streptozotocin that dissolved in 5 ml of normal saline per group was calculated by using the formula STZ (g) = 70mg/kg × total weight of animal in the group.

After an hour of post induction, the animal animals were continued on clean water and pelletized feeds.

After 72 hours following administration of STZ, fasting blood glucose was determined by glucometer (Accu-Chek Compact Plus). Blood was obtained from the median caudal vein of the tail by puncturing the tail. It was discovered that fasting blood glucose levels in the three induced groups was above 10 mmol/l [23], hence the animals were diabetic.

Treatment was commenced and the animals were treated for four weeks. The dose of the aqueous extracts of Cactus used in this study was adopted from the report of Saad [7]. Cactus was dissolved in physiological saline daily and was administered orally using an oro-gastric cannula to diabetes + cactus group rats (n = 6) at 100 mg/kg bw and cactus only group rats (n = 6) at 100 mg/kg bw for four weeks (at 9.00 - 10.00 a.m. daily). Diabetes + metformin group rats (n = 6) were administered 100 mg/kg bw of metformin at 9.00 - 10.00 a.m. daily for four weeks. Normal control and diabetes groups of rats (n = 6 each) received clean water and feeds only.

The following table shows the dosage of cactus and feeds.

Groups	Treatment	Duration
Normal control	Clean water and pelletized feeds	4 weeks
Diabetes (control)	Clean water and pelletized feeds	4 weeks
Diabetes + cactus	Cactus- 100mg/kg Clean water and pelletized feeds	4 weeks
Diabetes + metformin	Metformin- 100mg/kg Clean water and pelletized feeds	4 weeks
Cactus only	Cactus-100mg/kg Clean water and pelletized feeds Clean water and pelletized feeds	4 weeks

Table 1: Dosage (mg/kg body weight) and pelletized feeds.

Data collection

The blood glucose was measured weekly using a digital glucometer (Accu-Chek Compact Plus). Blood was obtained from the median caudal vein of the tail by snipping the tip of the tail. Body weight was taken on a weekly basis and recorded onto data entry sheet together with blood glucose level measurements for the entire experimental period. The relative weight of the brain was recorded after sacrificing the animals [26].

Animal sacrifice and histological process

At the end of four weeks of treatment, 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 the brain was carefully removed and weighed. The cerebellar tissues for histological studies were fixed in freshly prepared formo saline stored in specimen bottles for 72 hours. The tissues were processed for routine histological examinations stained with Haematoxylin and Eosin (H and E) and Phosphotungistic Acid Haematoxylin (PTAH) stains were used to observe changes in the cellular morphology.

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

Data analysis

The experimental data that was normally distributed for each group were expressed as mean ± standard error of the mean (mean ± SEM); analysed using one way ANOVA and all graphs were drawn using Excel (Microsoft Corporation, U.S.A). For all statistical tests a P values less than 0.05 ($p < 0.05$) was taken to be statistically significant. The histological analysis of cerebellar cortex was done using H and E and PTAH stains.

Research ethics

Ethics approval of the study protocol was granted by Mulungushi University School of Medicine and Health Science Research Ethics Committee (MUSoMHS-REC) (SMHS-MU2-2021-16). Standard requirements for the conduct of experiments on whole animals including 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.

Results

Average Body Weight Of The Rats(g)

Figure 1 shows the average body weight of the rats on weekly basis in different groups. (P < 0.05.) During the week of acclimatisation there was no significant difference in the average body weight in all the groups. At the end of the week of induction, the average body weights were almost as same as the week acclimatisation.

At the end of first week of treatment, there was an increase of 1.2g in the average body weight of the rats in diabetes group (183.5 ± 0.91g). By the end of the second week of treatment, there was further increase in the average body weights of diabetes group (184.07 ± 0.93g). In the last two weeks of treatment there was a decrease in the average body weights of diabetes group compared to the initial average body weight (182.1 ± 0.86g) at induction week. There was a significant difference in the average body weight (178.89 ± 0.74g) of diabetes group at week 4 when compared to the control group (188.74g (P < 0.05).

In the diabetes + cactus group, there was an increase of 2.33g in the average body weight of the rats at the end of week 2 of treatment (184.23 ± 0.91g) compared to induction week (182.01g). The average body weight further increased in the proceeding weeks and by the end of the week 4 of treatment (185.73 ± 0.93g) an increase of 3.72g was noted when compared with average weight at the end of week of induction (182.01 ± 0.80g) with no statistically significant difference when compared to the control group (188.74 ± 0.94g) (P > 0.05).

There was an increase in the average body weight of rats 1.81 in the diabetes + metformin group in at week 2 (183.91 ± 0.85g) when compare to the week of induction (182.1 ± 0.89g).

At fourth week of treatment, there was significant decrease (181.68 ± 0.80g) in the average body weight of diabetes + metformin when compared to the week of induction (182.1 ± 0.89g) with significant difference when compared to the control group (188.74 ± 0.94g) (P < 0.05).

The cactus only group mean body weight had no statistically significant difference when compared with the control group.

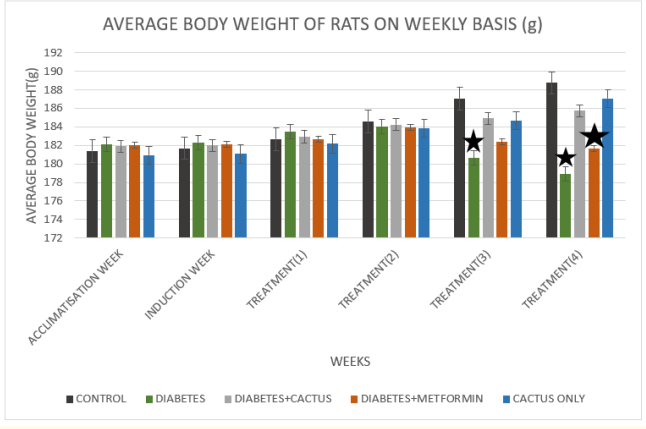

Figure 1: Histogram chart of average body weight on weekly basis. Data were expressed as Mean ± SEM. P < 0.05.

The relative organ weight

figure 2 below shows the relative weight of the brain of the rats in different groups. (P < 0.05). There was a significant decrese in the average brain weight in diabtes group (1.29g) as compared to the control group (2.09g) (P < 0.05).The relative weight of the brain in diabetes + cactus group(1.88g) and diabetes+ metformin group (1.76g) had no significant difference when compared with the control group (2.09g) (P > 0.05).

Average blood glucose level of the rats (mmol/L)

Figure 3 shows the average blood glucose levels of the rats on the weekly basis in various groups. (P < 0.05). At the acclimatisation week, the average blood glucose levels of the rats were within the normal range. At the end of the induction week, the three groups that were induced with diabetes had their glucose levels ranging between 23-29 mmol/L.

The diabetes group mean blood glucose remained hyperglycaemic from the end of induction period (23.6 ± 0.081) to the fourth week of treatment (33.7 ± 0.099). Diabetes + cactus group average blood glucose level was initially high (26.4 ± 0.083) after induction. However, at week 1 of the treatment, it decreased significantly

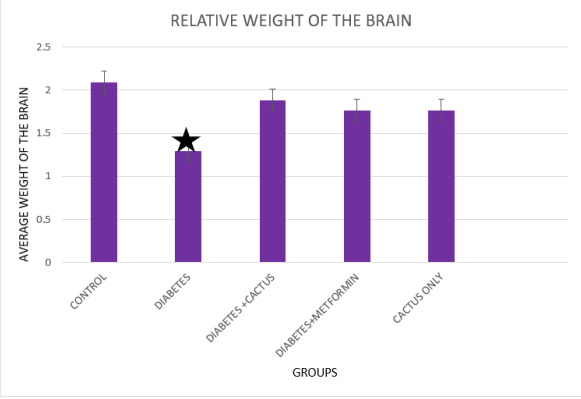

Figure 2: Histogram of relative weight of the brain. Data were expressed as Mean ± SEM. P < 0.05.

(19.1 ± 0.063) with further reduction at week 2 (10.6 ± 0.063). At fourth week, the mean blood glucose level in the diabetes + cactus group was within the normal range (5.9 ± 0.016) and no statistically significant difference was noted when compared with the control group (4.1 ± 0.018) (P > 0.05).

In diabetes + metformin group, the mean blood glucose level was (28.0 ± 0.088) after induction period. There was a relative decrease in average blood glucose level at week 1 (26.3 ± 0.081), with further decline at week 2 (12.5 ± 0.063). By the end of week 4, the average blood glucose level in diabetes + metformin group was (9.2 ± 0.032) indicating a significant reduction when compared to the induction period (26.4 ± 0.083) and was relatively high when compared to the control (4.1 ± 0.018) P < 0.05. The cactus only group mean blood glucose level was normoglycemic throughout the weeks.

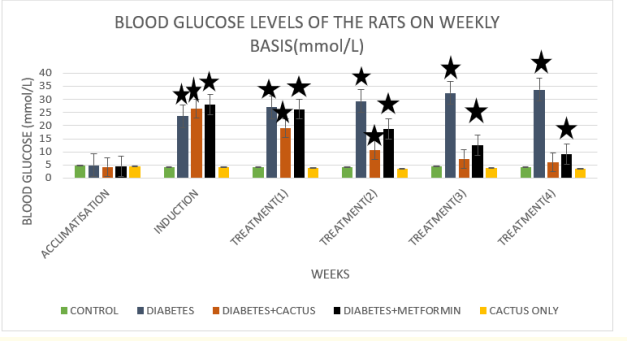

Figure 3: Histogram of blood glucose levels of rats on weekly basis. Data were expressed as Mean ± SEM. P < 0.05.

Histological findings

H and E stain

The normal control group showed the normal cerebellar cortex histoarchitecture of the molecular layer, granular layer and Purkinje cells (Figure 4 A). diabetic group showed numerous degenerating Purkinje cells (Figure 4 B). Cactus and metformin treated groups showed both normal Purkinje cells and degenerating Purkinje cells (Figure 4 C and D) while the cactus only group is similar to normal control group (Figure:4 E).

PTAH stain

The normal control group showed the normal histoarchitecture of the molecular layer, granular layer, Purkinje cells and Astrocyte (Figure:5 A). diabetic group showed numerous degenerating Purkinje cells with numerous astrocyte (Figure:5 B). Cactus and metformin treated groups showed both normal Purkinje cells and degenerating Purkinje cells and Astrocyte (Figure:5 C and D). While the cactus only group is similar to normal control group (Figure 5 E).

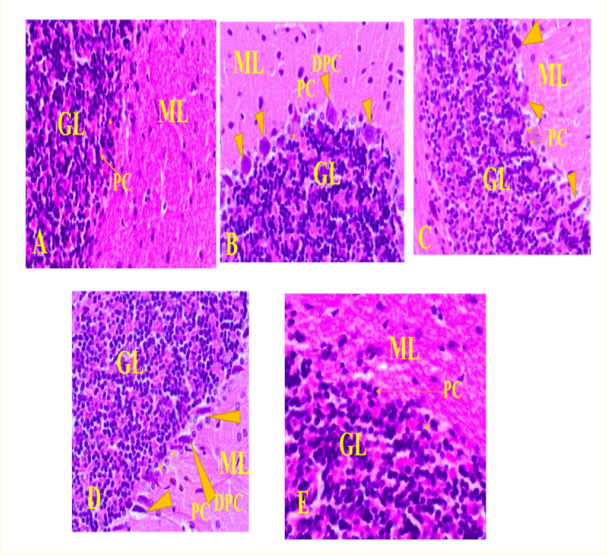

Figure 4: Photomicrograph showing the cerebellar cortex at day 28. H and E stain X400. A- Normal control, B - Diabetic, C - Diabetic + Cactus, D - Diabetic + Metformin and E- Cactus only. Arrow - Purkinje cells, arrowhead - Degenerating Purkinje cells, ML - Molecular layer, GL - Granular layer.

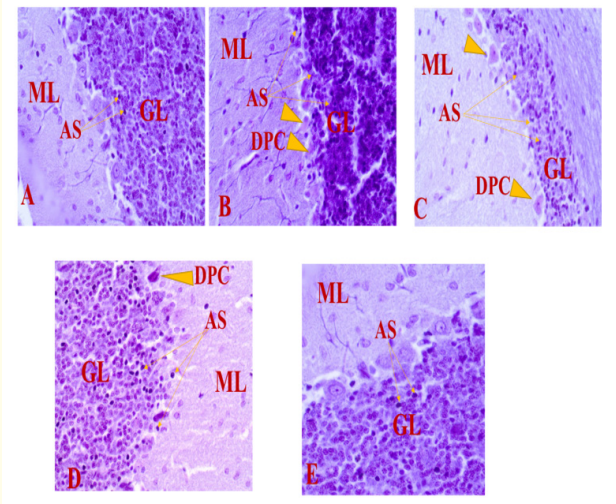

Figure 5: Photomicrograph showing the cerebellar cortex at day 28. PTAH stain X400. A- Normal control, B - Diabetic, C - Diabetic + Cactus, D – Diabetic + Metformin and E- Cactus only. Arrow - Purkinje cells, arrowhead - Degenerating Purkinje cells, ML - Molecular layer, GL - Granular layer, AS - Astrocyte.

Discussion

In the present study, the extract of cactus leaves was investigated for antidiabetic activity on diabetic Wistar rats, specific focus on the body weight, blood glucose level, relative organ weight (brain) and the histoarchictecture of the cerebrallar cortex. The observations were similar to many experimental diabetes researches [7,8]. The antidiabetic properties of cactus leaves is due to its phytochemical properties which include phenolics, Beta carotene, carotenoids and flavonoids [6]. Streptozotocin was chosen as a diabetes inducing agent because it was known to produced diabetes mellitus irreversibly with a single dose of intraperitoneal administration by relative necrotic action on the β cells of pancreas leading to insulin deficiency [4]. The destruction of β cells and disorders in insulin secretion in the diabetic state causes physio-metabolic abnormalities such as decreased weight, increase in food intake and water and increase blood glucose [8,26].

From the present study, we observed that streptozotocin-induced diabetes resulted in decreased body weight, which is according to AlFaris [21]. It is obvious that there was body weight gain in normal control group, followed by cactus only group and lastly by diabetes +cactus group. Highly significant decline in body weight was noted in diabetes group when compared to the normal control. These findings were consistent with other experimental diabetes investigations [25]. Consistent with our results, treatment of diabetic rats with cactus leaves extracts or metformin improves the body weight when compared to the diabetic control group which is in agreement with AlFaris [21]. The rats treated with cactus extracts only had body weight almost the same as the normal control.

The decrease in body weight is attributed to the increase of glucose with inhibition of insulin level, lipolysis, decline of tissue proteins and enhancement of muscle wasting in diabetes rats [18]. This Implies that the catabolic effects overridden the anabolic effects in diabetes group whilst anabolic effects were high in diabetes + cactus group [25]. Cactus leaves extracts administration at l00ml/kg to diabetic rats improved the body weight and this could be due to better hyperglycemic control in diabetic rats. These results are in harmony with Hwang [22] who reported that intraperitoneal injection of rats with streptozotocin caused highly significant decline in body weight gain compared to the normal group and oral administration of cactus extracts to diabetic Wistar rats improves the body weight significantly.

In the present study, it was shown that the relative weight of the brain of the rats decline significantly in diabetes group when compared to diabetes + cactus and the control group as indicated in figure 4.2. Metformin also maintains the relative weight of the brain in the diabetes rats, however, it is less effective than cactus leaves extracts. From the results obtained, cactus leaves extracts maintain the relative weight of the brain in diabetes rats within the normal range. This is attributed to the antioxidant properties (phenolic, flavonoids) contained in the cactus leaves which reverse or delay the metabolic complications that results in diabetes mellitus [16].

Diabetic rats were found to exhibit significant hyperglycemia as compared to normal control rats. In this study, cactus leaves extracts and synthetic diabetic drug (metformin) were used in the treatment of diabetes in Wistar rats. This study tested the effects of cactus on diabetes as it has been reported to decrease blood glucose in diabetic Wistar rats [11]. From the results, it was observed that there was a significant decrease in blood glucose levels in streptozotocin induced diabetic Wistar rats starting at week 1 to week 4 (Figure:3) in the group that received aqueous cactus extracts at 100ml/kg compared to diabetes group and showed more reduction in blood glucose level compared to the group that re-

ceived metformin. By week 4, the blood glucose level in diabetic + cactus was normoglycemic while in metformin was hyperglycemic. This indicates that cactus extracts are more effective in the treatment of diabetic rats than metformin. The group administered with aqueous cactus extracts only remained normoglycemic throughout the experiment. This could indicate that aqueous cactus leaves extracts do not have hypoglycemic effects in non- diabetic Wistar rats. These findings are consistent with other existing findings [14] that similarly reported the antidiabetic activity of other species of cactus extracts in other setups.

In the histopathological investigation of the present study, the cerebellar cortex of control group and cactus only group (Figure 4A, 4E, 5A and 5E) showed similar histoarchitecture. The histopathological examination of the diabetes groups (Figure 4B and5B) showed numerous degenerating Purkinje cells. Diabetes reduces the Purkinje cell density. This is attributed to high glucose levels in the cell which stimulate increased oxidative stress through auto oxidation of high intracellular glucose, with unstable glycation, activation of polyol pathway and endogenous depletion of antioxidants. The generation of excess free radicals contributed to increased neuronal death by oxidation of proteins, and activation of cellular lipid peroxidation [20]. In diabetes + cactus group, the photomicrographs (Figure 4C and 5C) showed improved and less degenerative Purkinje cells and astrocyte. This is in agreement with El-Mostafa., *et al.* (2014) who reported that cactus extracts prevent inflammation and neurodegeneration. This is because cactus contain substantial amount of antioxidant compounds (phenols, flavonoids, betaxanthin and betacyanin) which have hypoglycaemic and antioxidants properties. The photomicrographs (Figure 4D and 5D) of diabetes + metformin exhibited improved and much less degenerating Purkinje Cells than diabetes + cactus. It has been used as a synthetic oral anti-diabetic drug, it does not only have hypoglycaemic, but also antioxidant properties. This is in agreement with Piasecka [17] who reported that metformin ameliorates the oxidative damage in diabetes.

Conclusion

Aqueous cactus extracts significantly reduced the blood glucose levels in the streptozotocin induced diabetes Wistar rats as compared to the conventional oral antidiabetic drug metformin used in the management of diabetes. It has also been shown that aqueous cactus extracts maintain the body weight and improves the cytoarchitecture of the cerebellar cortex by reversing or halting the metabolic effects on Purkinje cells in diabetic rats which is attributed to the antioxidants such phenols and flavonoids contained in cactus. These findings suggest that cactus should be subjected to clinical trials in humans.

Bibliography

1. Aastha Chawla., *et al*. "Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum?" *Indian Journal of Endocrinology and Metabolism* 20.4 (2016): 546-551.

2. Adeyi Akindele O., *et al*. "Amelioratie potentials of medicinal plants on the pathophysiological complications of diabetes mellitus". *Journal of Medicinal Plants Research* 9.8 (2015): 262-288.

3. Adenowo TK., *et al*. "Neurobehavioural study on the effect of aqueous extract of Citrus medica leaf on prefrontal cortex of hyperglycemic wistar rats". *Journal of Molecular Histology and Medical Physiology* 3 (2018): 1.

4. Ajay Kumar Sharma and Ritika Gupta. "Anti-Hyperglycemic Activity of Aqueous Extracts of Some Medicinal Plants on Wistar Rats". *Journal of Diabetes and Metabolism* 8 (2017): 7.

5. Anastasia Poznyak., *et al*. "The diabetes mellitus- atherosclerosis connection: the role of lipid and glucose metabolism and chronic inflammation". *International Journal of Molecular Sciences* 21.5 (2020): 1835.

6. Anhar M Gomaa., *et al*. "Effects of cactus pear (Opuntia ficus indica) juice on oxidative stress in diabetic cataract rats". *Saudi Journal for Health Sciences* 1.1 (2012): 23.

7. Anouar Ben Saad., *et al*. "Phytochemical, antioxidant and protective effect of cactus cladodes extract against lithium-induced liver injury in rats". *Pharmaceutical Biology* 55.1 (2017): 516-525.

8. Atef M Al- Attar., *et al*. "Effects of Olea europaea leaves extract on sztreptozocin induced diabetes in male albino rats". *Saudi Journal of Biological Sciences* 5 (2019): 23-30.

9. CS Wu., *et al*. "Association of depression and diabetes complications and mortality: a population-based cohort study". *Epidemiology and Psychiatric Sciences* 29 (2020): e96.

10. Fatmah A Matough., *et al*. "The role of oxidative stress and antioxidants in diabetes complications". *Sultan Qaboos University Medical Journal* 12.1 (2012): 5.

11. Hyunmu Jo., *et al*. "Association between extracts from fruit of Optuntia ficus indica and streptozocin induced diabetes rats". *Korean Journal of Veterinary Research* 61 (2021): 1.

12. Iizeus Rutebemberwa., *et al*. "Use of traditional medicine for the treatment of diabetes in Eastern Uganda; a qualitative exploration of reasons for choice". *International Health and Human Rights* 13 (2013): 1.

13. International diabetic forum. IDF diabetes atlas 9th edition (2019).

14. Kalungia C Aubrey., *et al*. "Opuntia stricta cladode extracts reduces blood glucose levels in alloxan induced diabetes mice". *International Journal of Diabetes in Developing Countries* 7.1 (2018): 1-11.

15. Karym El- Mostafa., *et al*. "Nopal cactus (Optuntia ficus indica) as a source of bioactive compounds for nutrition, health and diseases". *Mol* 19.09 (2014): 14879-14901.

16. Jangampalli A Pradeepkiran., *et al*. "Trigonella foeonum graecum seeds extract plays a beneficial role on the brain antioxidant and oxidative status in alloxan induced wistar rats". *Food Quality and Safety* 4 (2020): 83-84.

17. Magdalena Markowicz-Piasecka., *et al*. "Metformin a future therapy for neurodegenerative diseases". *Pharmaceutical Research* 34 (2017): 2614-2627.

18. Muhammad A. "Abdul-Ghani and Ralph A. DeFronzo (2010). Pathogenesis of Insulin Resistance in Skeletal Muscle". *Journal of Biomedicine and Biotechnology* 476279 (2010): 19.

19. Nitin Chaudhary and Nidhi Tyagi. "Medicinal plants used for diabetes mellitus diabetes mellitus; an overview". *International Journal of Research and Development in Pharmacy and Life Sciences* 7.4 (2018): 3022-3029.

20. Niyomchan A., *et al*. "Cerebellar sypnaptopathy in streptozocin induced rats". *International Journal of Morphology* 37.1 (2019): 28-35.

21. Nora A Alfaris., *et al*. "Antidiabetic and antihyperlipidemic effect of *Duvalia corderoyi* in rats with streptozocin induced diabetes". *Saudi Journal of Biological Sciences* (2020).

22. Seung Hwan Hwang., *et al*. "Antidiabetic effect of fresh nopal (*Opuntua ficus indicain*) low dose streptozocin induced diabetic rats fed a high fat diet". *Evidence-Based Complementary and Alternative Medicine* 2017.438017 (2017): 8.

23. Susan Gottlieb and Jacquie Rand. "Managing feline diabetes: current perspectives". *Veterinary Medicine (Auckland, N.Z.)* 9 (2018): 33-42.

24. Tahsini Lachin and Heydari Reza. "Anti-Diabetic Effect of Cherries in Alloxan Induced Diabetic Rats". *Recent Patents on Endocrine, Metabolic and Immune Drug Discovery* 6 (2011): 67-72.

25. Waleed Hassan Almalki., *et al*. "Role of Phyto Nutraceuticals in Management f Hyperglycemia". *Natural Volatiles and Essential Oils* 8.6 (2021): 3523-3531.

26. Yusuf U., *et al*. "Histological study on the effects of aqueous extracts of citron leaf on pancreas of hyperglycemic wistar rats". *Biological Sciences and Pharmaceutical Research* 7.5 (2019): 82-90.