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Micro anatomical effects of aqueous cactus extract in the lungs of a diabetic Wistar rat

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Abstract

Opuntia stricta is a naturally occurring plant and is readily available in some parts of Zambia where it is used for its decorative nature and its fruits consumed. This present laboratory based study was carried out to assess some micro anatomical effects of aqueous cactus on the lungs of a diabetic Wistar rat. The male adult Wistar rats (30) weighing between 160-200 g were indiscriminately classified into five groups consisting of 6 Wistar rats namely: control, diabetic control, Diabetes+ Metformin, Diabetes+ cactus and cactus only groups. Diabetes was induced with Streptozotocin dose 70 mg/kg BW and sustained increase in blood glucose levels after 72 hours and treatment commenced with aqueous cactus extract and Metformin with 100 mg/kg body weight respectively and was administered orally for 4 weeks . Blood glucose levels (mmol/L) were monitored. After 4 weeks of treatment the animals were sacrificed by euthanasia, the lungs were excised, weighed and fixed in 10% formal saline. Data was analyzed using Excel and one way ANOVA. $p<0.05$ was considered significant. Change in body weight and relative organ weight of the Diabetic+ cactus and Diabetes + Metformin was significant when compared to diabetic group($p<0.05$). Diabetic + cactus became normoglycemic at week 3 and diabetic+ Metformin at week 4. The histological examination of the lungs of the diabetic rats showed disruption in cytoarchitecture with necrotic pneumocyte. Diabetic + cactus and Diabetes + Metformin showed no disruption in their cytoarchitecture and both had healthy pneumocyte present. Cactus only was similar to control. In conclusion, aqueous extract of Cactus showed ameliorative effects on the lung of adult male Wistar rats against the damage caused by diabetes.

Keywords: Lungs; Streptozotocin; Aqueous cactus extract; Diabetic; Wistar rat

1. Introduction

Diabetes mellitus is a group of metabolic diseases with a common feature of hyperglycemia, it is associated with inflammation and oxidative stress which leads over time to serious damage to the vascular system. It may be due to insulin deficiency or lack of response of the body cells to insulin or both. It affects more than 120 million people and is estimated that over 370 million people will be affected by 2030.The clinical symptoms include polyuria (increase in urine frequency), polydipsia (increased thirst) and polyphagia. People with diabetes mellitus may live a normal healthy lifestyle of course with complications here and there despite the fact that it is irreversible. The common types of diabetes are Type 1, Type 2 diabetes and gestational diabetes (1).

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Type 1 diabetes is an autoimmune condition characterized by pancreatic beta cell destruction and an absolute deficiency of insulin. It is responsible for 5% to 10% of all cases. It is mostly diagnosed during childhood but may also be diagnosed during adulthood known as Latent Auto immune Diabetes in Adults (1).

Type 2 diabetes on the other hand comes about when the body tissues develop insulin resistance. With time the levels of blood sugar keep rising leading to a number of symptoms that include; polyphagia, polydipsia and weight loss, fatigue, blurred vision, slow healing sores. According to WHO majority of individuals have developed type 2 diabetes as a result of modifiable risks such as obesity and sedentary lifestyle. Additional risk factors include; family history, blood lipid levels, pre diabetes (2).

There is no cure for diabetes, but leading a healthy lifestyle, eating well, exercising and compliance to medication can help manage the disease. The late complications of diabetes lead to increased risk of mortality and major health costs. These include micro vascular damage causing diabetic nephropathy, retinopathy and neuropathy being the major complication chronic hyperglycemia predisposes an individual to an increased risk of cardiovascular diseases, diabetes ketoacidosis common individuals with type 1 diabetes, neuropathy, nephropathy and retinopathy. Broad studies on the above mentioned complications have been undertaken over the years. However, little work has been done on the concept of the lung as a target organ (3).

Medicinal plants including the prickly pear cactus (*Opuntia stricta*) is a natural plant grown in some parts of Zambia especially the dry ones. It bears fruits and cladodes consumed for medicinal and nutritional purposes. Prickly pear cactus has been identified as one of the modulators of blood sugar levels. There is scanty evidence whether *opuntia stricta* extract indigenously grown in Zambia has antidiabetic effect (4,5). Extracts of prickly pear cactus are being used in various parts of the world to manage diabetes mellitus. In certain parts of Africa like Kenya cactus is being viewed as a weed (6). Therefore, this study will be aimed at assessing the efficacy and pharmacokinetics of prickly pear cactus extracts from Zambia in managing diabetes mellitus induced in mice.

2. Material and methods

2.1. Plant materials

The Cactus was harvested from Livingstone district, Southern Province of Zambia, then subjected to identification at the University Of Zambia School Of Natural Sciences under the Department of Biological Sciences before the study begins. The Cactus was air dried and pounded. The dry pounded Cactus was then be ground and sieved to obtain a homogenous powder. The extraction was done using (5, 7) methods.

2.2. 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 (ad libitum).

2.3. Induction of diabetes

Streptozotocin was used to induce diabetes. The rats were weighed, and a baseline glucose level established after the overnight fasting period. The animals were injected calculated at a dose of 100 mg/kg body weight and reintroduced to the normal feeding cycle (5, 7, 8). It takes 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. Animals were considered diabetic with fasting blood glucose levels above 7 mmol/l.

2.4. Experimental design

Thirty adult healthy male Wistar rats were randomly 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 Cactus extract, Group C was diabetes treated with Cactus extract only, Group D was diabetes treated with Metformin and Group D was cactus extract only (7,8)

2.5. Cactus mode of administration

The dose of the aqueous extracts of Cactus used in these studies was adopted from the report (8,9). 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 (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, Group E rats (n=6) were administered 100 mg/kg bw of Cactus extracts. Group A rats (n=10) received neither streptozotocin nor Cactus extract (8).

2.6. Measurement of blood glucose level

The blood glucose levels were 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 (acclimatisation period) before the induction of Diabetes and for four weeks of treatment (8, 10).

2.7. 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); (7,10).

2.8. 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); (8,10).

2.9. Histological process

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 were carefully removed and weighed. The tissue for histological studies were fixed in freshly prepare formal saline for 72 hours and processed for routine histological examinations stained with Hematoxylin and Eosin (H&E) to observe changes in the cellular morphology.

2.10. Photomicrography

Photomicrography of histological sections of the lung were 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, Zambia.

2.11. Statistical analysis

Data were presented as mean ± standard error of the mean (mean±SEM); analyzed using one way ANOVA and all graphs will be 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 the average body weight changes taken weekly of Wistar rats belonging to various groups. After week of acclimatization all groups were not significant ($p>0.05$) when compared to the control group. In week 3 there was significant decrease in the diabetic group when compared to the control ($p<0.05$), diabetic+ cactus and cactus only groups which are significant ($p<0.05$) with diabetic group. In week 4 diabetic group and diabetic+ Metformin continue showing a decrease and when compared to control was statistically significant ($p<0.05$).

Figure 2 shows changes in blood glucose levels of various groups of Wistar rats taken weekly. During the week of acclimatization the blood glucose levels of all groups were normal and when compared to the control group. During the week of induction (week 0), the diabetes, diabetes+ cactus and diabetes+ Metformin showed an increase in blood glucose levels respectively. In week 1 of treatment diabetes + cactus showed a significant decrease when compared to diabetes and diabetes+ Metformin which were significant $P<0.05$. Diabetes group remained significantly different ($p<0.05$) to other groups up to the fourth week of treatment. Diabetes + Metformin and Diabetes + cactus were not significant ($p>0.05$) in week 3 and 4 when compared to control group.

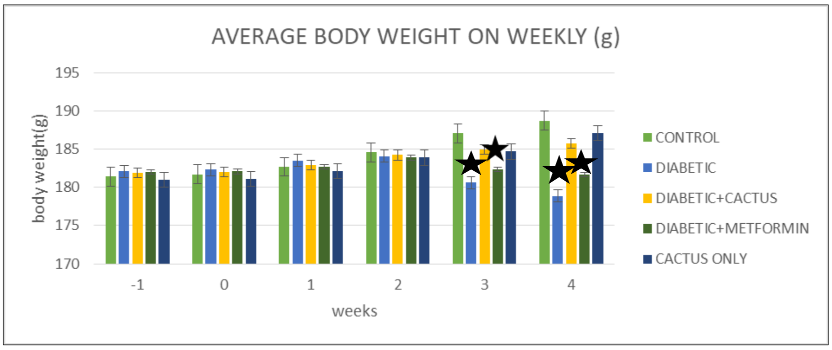

Figure 1 Average body weight on weekly. Data were expressed as mean ± SEM ($p<0.05$) * asterisk means significance at $p<0.05$

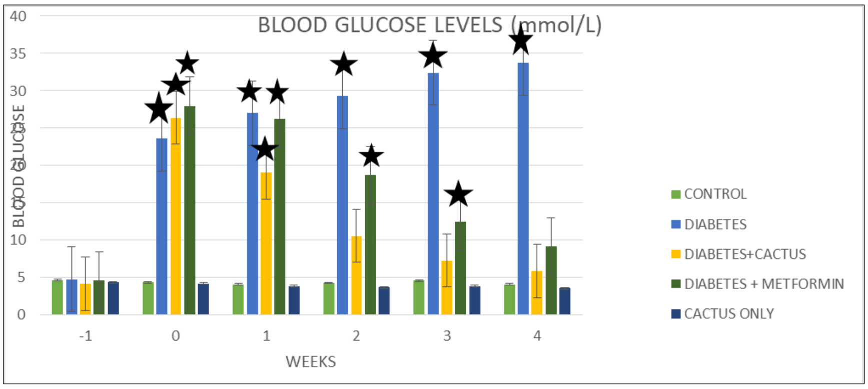

Figure 2 Changes in blood glucose levels. Data were expressed as mean ± SEM ($p<0.05$) * asterisk means significance at $p<0.05$

Figure 3 shows the relative organ weight of the lung in various groups. There was a significant decrease in weight in the diabetes group (0.65) g, diabetes + cactus (1.21) g, diabetes +Metformin (1.09) g when compared to control and cactus group (1.45 and1.48) g respectively.

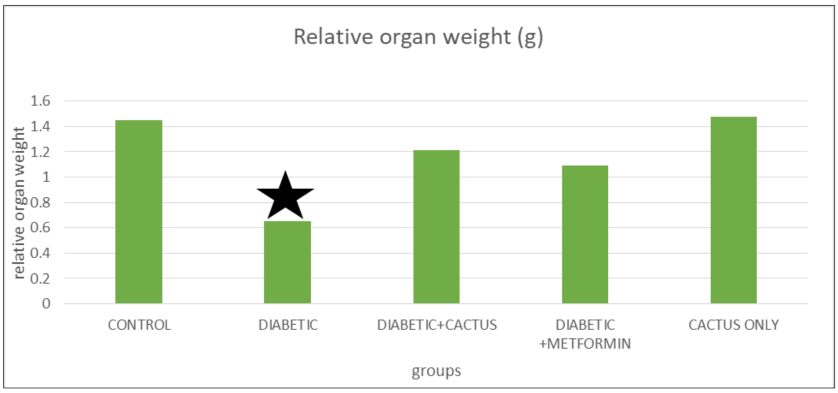

Figure 3 Relative lungs weight in various groups. Data were expressed as mean ± SEM ($p<0.05$) * asterisk means significance at $p<0.05$

3.1. Histology of the lungs

The lungs in the normal control group showed normal cytoarchitecture with numerous healthy pneumocyte, (Figure : 4 A). Diabetic group showed the cytoarchitecture was disrupted with necrotic pneumocyte (Figure 4 B). Cactus and

Metformin treated groups showed no disruption in their cytoarchitecture and there are both healthy pneumocyte present (Figure: 4 C and D). Cactus group is similar to normal control (Figure 4 E)

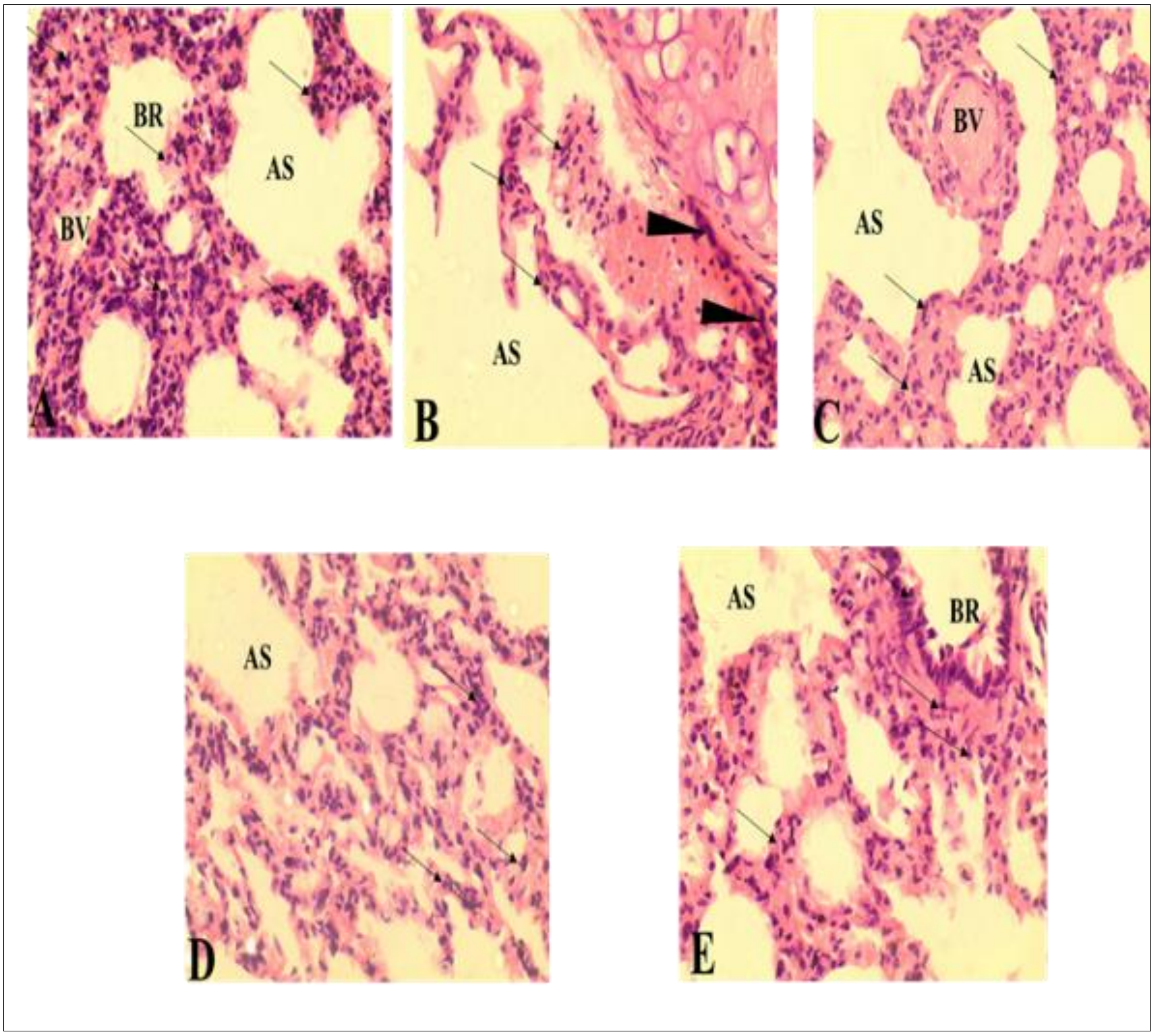

Figure 4 Photomicrograph showing the Lungs at day 28. H&E stain X400. A- Normal control, B – Diabetic, C – Diabetic+Cactus, D – Diabetic+Metformin and E- Cactus only. AS – alveolar sac, BR – Bronchioles, BV – Blood vessel, arrow – pneumocyte, arrow head necrotic pneumocyte

4. Discussion

A prolonged state of hyperglycemia is an important diagnostic indicator of diabetes mellitus. Current scientific evidence on Cactus (*Opuntia stricta cladodes)* demonstrates phytochemical constituents that contribute to potential hypoglycemic effects (11). In this study, an aqueous extract of cactus was used to ascertain the microanatomical effects on the lungs of diabetic Wistar rats.

In this study the average body weight of each group of rats was recorded and used to evaluate the changes weekly. It was observed that during induction all the rats in each group had a similar weight range. The average body weights began to change during the first week of treatment. The diabetic+ cactus and diabetic + metformin groups maintained a good average body weight in week 3 when compared to the control group but in week 4 diabetic + cactus group increase in average weight while diabetes+ metformin decreased. The diabetic group decreases both in week 3 and 4, when compared to other groups it was significant $P<0.05$. This could be attributed to lipolysis and increased protein breakdown. Present finding of this study is in agreement with (10, 12).

This present study showed the blood glucose levels in diabetic group Wistar rats revealed a marked hyperglycemic state compared to cactus and control groups (figure 2). The findings showed that the rats treated with cactus had a rapid decrease when compared to the diabetic rats treated with metformin. The Wistar rats treated with cactus became normoglycemic at week 3. It can be attributed to the high content of compounds such as soluble (mucilage and pectin) and insoluble fiber (hemicellulose and lignin), polyphenols and vitamin C. The presence of all these compounds makes a low glycemic index and foods with a low index tent to release glucose slowly into circulation by decreasing the absorption of sugar in the stomach and intestine and this helps in keeping the glucose levels under control. This is in agreement with (5, 8).

It was observed that the relative organ weight of the treated rats with either cactus or metformin had increased when compared to the diabetic group rats this might due necrotic of the cells seen histologically in this present study and it has been reported by (7) that hyperglycemia could induce necrosis in addition to oxidative stress by inducing the emergence of reactive oxygen species (ROS) and imbalance between oxidant and antioxidant species. The Wistar rats treated with cactus had a greater relative organ weight than the ones treated with metformin but both are not significant to control group rats.

According to the histological images obtained the normal control group showed normal cytoarchitecture with numerous healthy pneumocytes. All the groups when compared to the control group showed no disruption except the diabetic group which had disrupted cytoarchitecture with necrotic pneumocyte. Buildup of oxidative stress plays an important role in the pathogenesis of diabetes and it is of this reason that the pneumocytes underwent necrosis on the hand lungs of the rats treated with cactus and metformin did not undergo necrosis due to the presence of antioxidants such as phenolic acids, flavonoids and pigments This is in agreement with (8,11)

5. Conclusion

Aqueous extract of cactus was able to reduced blood glucose levels much quicker than Metformin and also ameliorate the disruption caused by diabetes mellitus on the histoarchitecture of the lungs.

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 ethic committee

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