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Streptozotocin and diabetes: Modulatory role of an aqueous extract of cactus on kidney histo-architecture of model

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Abstract

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The prevalence of DM globally in 2019 was estimated to be 9.3% of the world's population. Complications of DM include nephropathy, neuropathy, retinopathy, stroke and coronary heart disease. Traditional plants have been used in the management of diabetes mellitus, an example is the cactus plant. This study aimed to shade more light on the effect of an aqueous extract of cactus on the kidney histo-architecture of the diabetic Wistar rats. Thirty adult male Wistar rats weighing between 160-200g and were randomly grouped into five consisting of six rats each: group A. normal control, group B. diabetic only, group C. diabetic treated with Cactus and group D. diabetes treated with Metformin only and group 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≥ 7mmol). The cactus extract and Metformin was administered orally at 100 mg/kg body weight daily for four weeks and blood glucose level were recorded weekly. After the fourth week of administration, animals were sacrificed by euthanasia. The kidneys from all the groups were weighed and their weight recorded and were fixed in 10% formal saline. Data was analyzed using one way ANOVA $p<0.05$ was considered significant and the graphs were plot using excel. 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 Diabetic +Cactus group and diabetes+ Metformin when compared to the diabetic group was significant $P<0.05$. Histologically, the diabetic group that showed disruption of macula densa and a large urine space compared to the Wistar rats in the Diabetes+cactus group and Diabetes+metformin group that showed little disruption in macula densa were observed. From the results obtained, the aqueous cactus extract has the ability to lower blood glucose level in diabetic Wistar rat and is histologically renoprotect to the damage caused by Diabetes mellitus.

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Keywords: Diabetes; Cactus; Streptozotocin; Wistar rat; Metformin

1. Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1].The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

There are two main types of diabetes mellitus and the others, the main types of diabetes mellitus include type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes mellitus [2].

Type 1 diabetes mellitus occurs as a result of failure of the body to produce insulin, it has a predominant onset in the childhood age group and needs insulin administration to manage it that is why it was previously called insulin-dependent, juvenile or childhood onset, in this type of diabetes the predisposing factors and preventive measures are not known. Type 2 diabetes mellitus is as result of failure of the body to utilize insulin, this type of diabetes has a predominant onset in adulthood age group. Majority of people who have diabetes have type 2 diabetes, the common predisposing factors are overweight and physical inability. Gestational diabetes is an increase in blood glucose levels above normal but cannot be considered as diabetes mellitus, which occurs during pregnancy but may resolve after delivery of the baby. These women and likely their children are also at an increased risk of developing type 2 diabetes in the future [2].

The global prevalence of diabetes mellitus in 2019 was estimated to be 9.3% [463 million people], of which it is likely to rise to 10.2% [578 million] by 2030 and 10.9% [700 million] by 2045. The prevalence of diabetes mellitus is higher in urban [10.8%] than rural [7.2%] areas, and in high-income [10.4%] than low-income countries [4.0%] [2].

The classic symptoms diabetes mellitus include increase in the frequency of urination, excess thirst and increase in food intake, fatigue, weight loss and increased frequency of infection [3].

Diabetes mellitus is accompanied by long term complications that are particularly specific to diabetes mellitus these include neuropathy, retinopathy and nephropathy, these complications occur in both type 1 and 2 diabetes mellitus, apart from these complications diabetes mellitus is also accompanied by atherosclerotic disease of blood vessels which include blood vessels of the heart, the brain and the peripherals which results in coronary heart disease, stroke and diabetic foot disease [3].

In the management of diabetes mellitus synthetic drugs such as insulin can be used alongside traditional plants.

Cactus plant of Opunita sp. Also known as pricky pear or nopal is a member of cactaceae family. Cactus plant is known by different names based on the interior and exterior colour, size and region of production [4]. the cactus plant is originally from central America and the Caribbean islands but can be found in other parts of the world including Europe and Africa [5].

Cactus plant is known for treating diabetes, high cholesterol, obesity and hangover, this is due to its anti-inflammatory, antioxidants and carotenoids [4]. Prickly pear cactus is popular in many areas of the world. The edible parts are the leaves, flowers, stems and fruit [6,7]Cactus is eaten whole [boiled or grilled] [6,7]. It is also made into juice and jams.

2. Material and methods

2.1. Plant materials

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 grounded and sieved to obtain a homogenous powder. Yusuf et al., [8] method was used for the aqueous extraction of the powder.

2.2. Animals and animal management

Thirty adult presumably healthy male Wistar rats were used in the study of which were divided into five groups that were named control group, diabetes+ metformin group, diabetes+ cactus group and cactus only group. The animals were between 8 to 10 weeks old; body weight was between [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.

2.3. Induction of diabetes

Streptozotocin was used to induce diabetes in the Wistar rats. The animals were acclimatized for one week and blood glucose levels measured. They were weighed, and a baseline glucose level was established after the feeds was withdrawn overnight period [18:00-06:00]. The animals were injected intraperitoneally with streptozotocin of 70 mg/kg body weight and reintroduced to the normal feeding cycle [8, 9]. After 72 hours after induction, a fasting blood sugar were collected to determine the establishment of diabetes using the tail vein puncture, using a glucometer to access blood glucose levels. Animals were considered diabetic with fasting blood glucose levels above 7 mmol/L or ≥ 250 mg/dL.

2.4. Experimental design

Thirty adult healthy male Wistar rats were divided into five groups of six [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.

2.5. Cactus mode of administration

The dose of the aqueous extracts of Cactus used in the study was adopted from the report by Saad [10]. Cactus was dissolved in physiological saline daily and was administered orally with use of oro-gastric cannula to Group C rats at 100 mg/kg body weight of cactus extract [at 9.00 – 10.00 a.m. each day] for a maximum period of four weeks, Group D at 100 mg/kg body weight of metformin, Group E rats were administered 100 mg/kg body weight of cactus extracts. Group A rats received neither streptozotocin nor cactus extract [7].

2.6. Measurement of blood glucose

The blood glucose was evaluated in overnight fasted rats at 9:00 – 10:00 hours using glucometer. Blood was obtained from the vein of the tail by snipping the tip of the tail. The blood glucose level was monitored weekly from acclimatization period before the induction of Diabetes and for four weeks of treatment [8, 9].

2.7. Measurement of the body weight (g)

Body weight (g) of the rats was recorded for one week of acclimatization and on weekly basis during the experimental treatment for a period of four weeks. Body Weight was taken with a weighing scale [8, 11, 12].

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]; [11,8].

2.9. Histological process

At the end of the 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 abdomen of the animals were dissected with foreceps and each kidney was carefully removed and weighed. The tissues for histological studies were fixed in freshly prepare 10% formal saline for 72 hours and processed for routine histological examinations stained with Haematoxylin and Eosin [H&E] and Masson.

2.10. 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

2.11. Data analysis

Data was presented 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]. P values less than 0.05 [p<0.05] was taken to be statistically significant.

3. Results and discussion

3.1. Average body weight on weekly basis

Figure 1 shows the weekly changes in body weight in the different groups. The body weight of the rats in the different groups in the week of acclimatization were normal with no significant difference when compared with the control. After induction and initiation of treatment the change in the body weights of the various was not enough when compare to the control group until week three of treatment, that is when the reduction in weight was noted in the diabetes control group [p>0.05] compared to those in the control group and those that were being treated with cactus and metformin. But there was no significance in the cactus only group when compared to those in the control group by the fourth week of treatment.

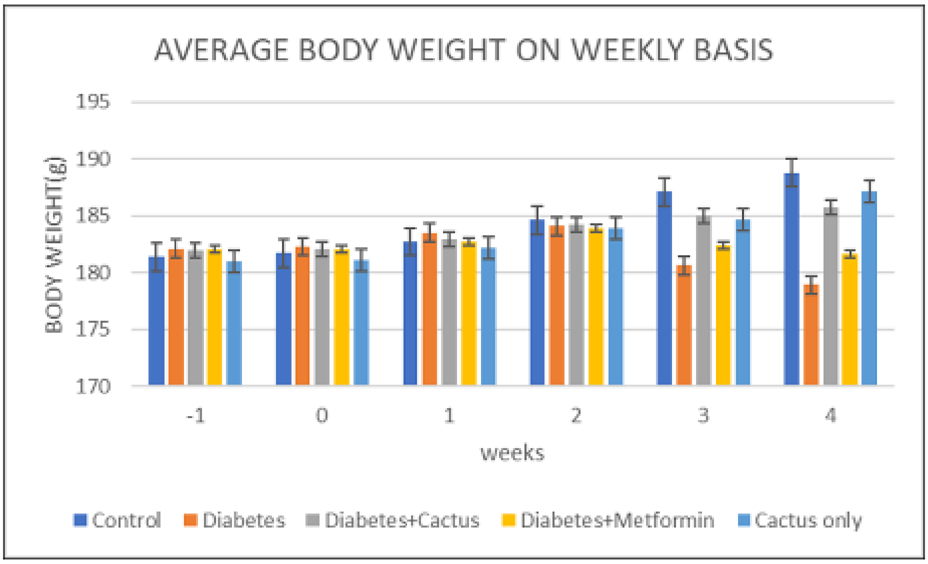

Figure 1 Average Body weight [g] On Weekly Basis. Data were analysed using mean ± SEM and p<0.05 considered significant

3.2. Average blood glucose levels on weekly basis

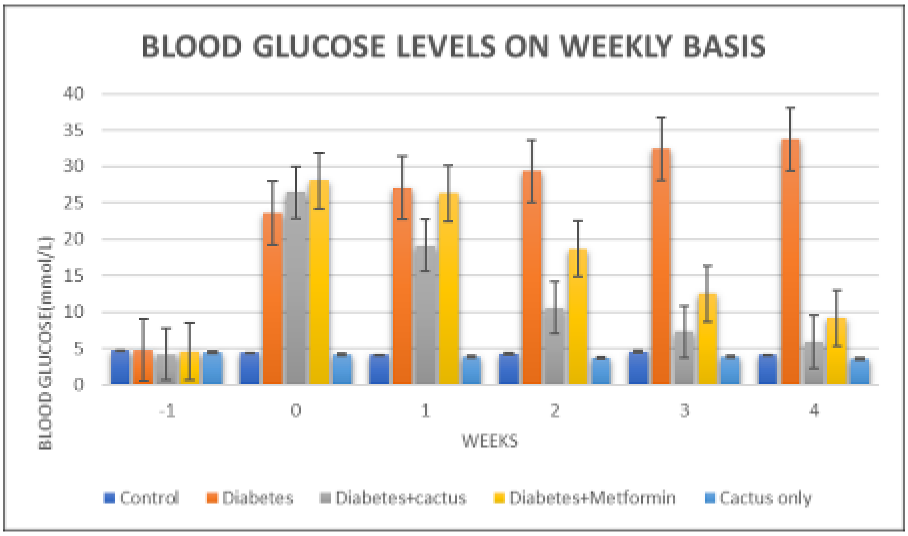

Figure 2 Blood Glucose Levels [mmol/L] On Weekly Basis. Data were analysed using mean ± SEM and p<0.05 considered significant

Figure 2 shows the levels of blood glucose of the various groups of rats on weekly basis. The blood glucose levels of the various rat groups were normal in the week of acclimatization with no significant different when compared to the control group [p>0.05]. After induction and initiation of treatment, the rats in the diabetes control group were hyperglyaemic and remained hyperglycemic until the end of treatment but the rats in the groups that were being treated with cactus and metformin were significantly high when compared to the control group but by second week up to the last week of treatment there was significant difference when compared to the diabetes control group.

3.3. Relative organ weight

Figure 3 shows the relative weight of the kidney in the various group of rats. There a significant decrease in the weight of the kidney of the rat in the diabetes control group when compared to those in the control group. When compared to those that were in the diabetes plus cactus group to those in the diabetes plus metformin group there was no significant difference in the weight of the kidney.

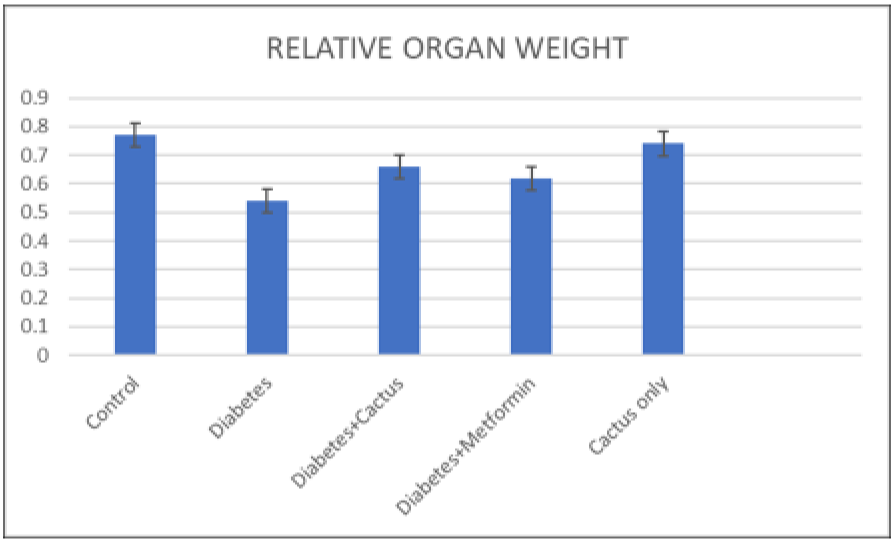

Figure 3 Relative Organ Weight. Data were analysed using mean ± SEM and p<0.05 considered significant

3.4. Histology of the kidney

The kidney in the normal control group showed normal histoarchitecture with glomerulus, urine space and macula densa [Figure 1A]. diabetic group showed disruption of macula densa and urine space is large [Figure 1 B]. Cactus and metformin treated groups showed little disruption in macula densa and urine space is observed large [Figure: 1 C and D]. cactus group is similar to normal control [Figure 1 E]. the normal control showed normal distribution of collagen [Figure 2 A]. Diabetic group showed a lot of deposition of collagen [Figure 2 B]. Cactus and metformin treated groups showed a little deposition of collagen [Figure 2 C and D]. Cactus only group was similar in histoarchitecture with the normal control group [Figure 2E].

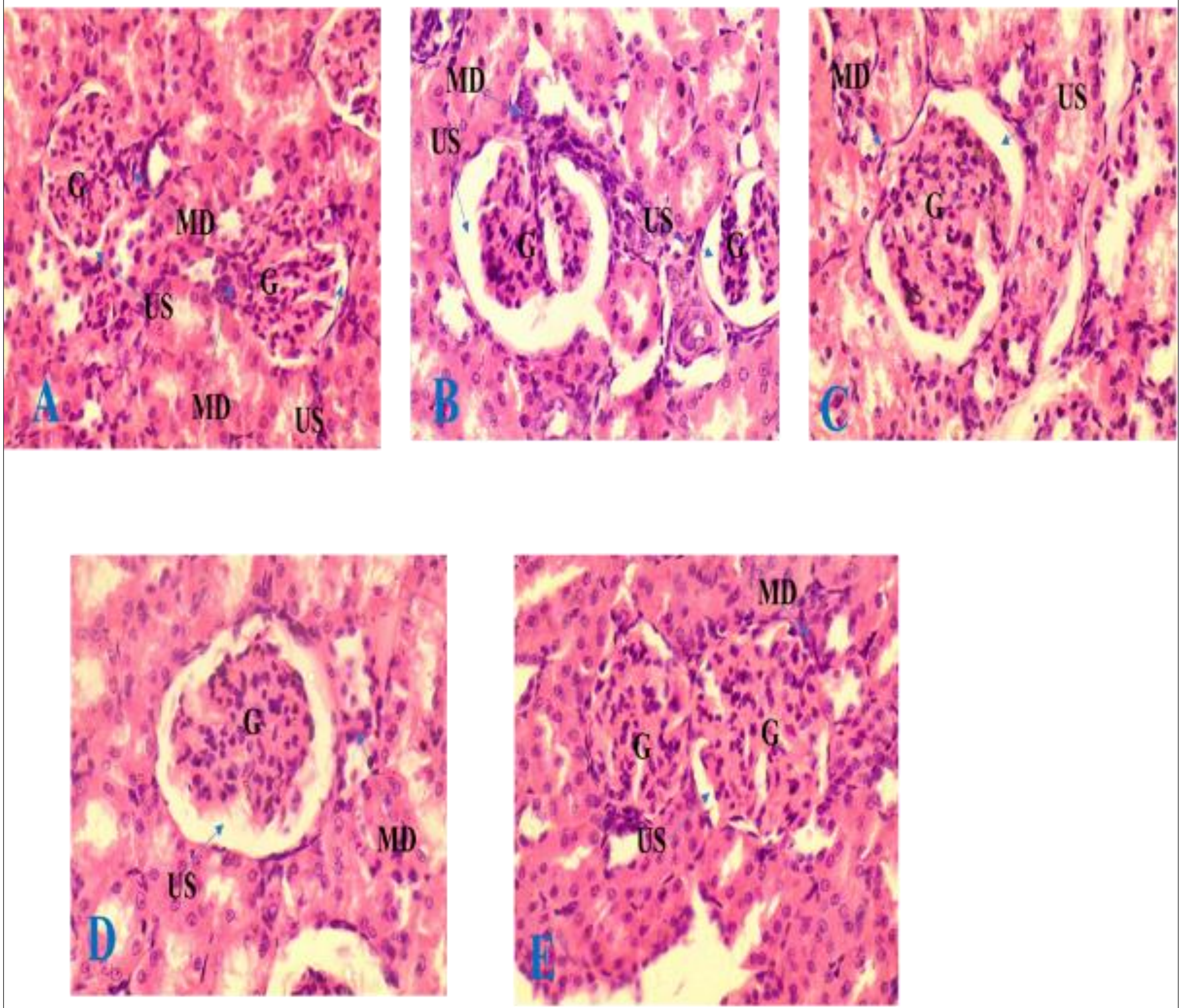

Figure 4 Photomicrograph showing the Kidney at day 28. H&E stain X400. A- Normal control, B – Diabetic, C – Diabetic+Cactus, D – Diabetic+Metformin and E- Cactus only. G - glomerulus, MD – Macula Densa, US – Urine space

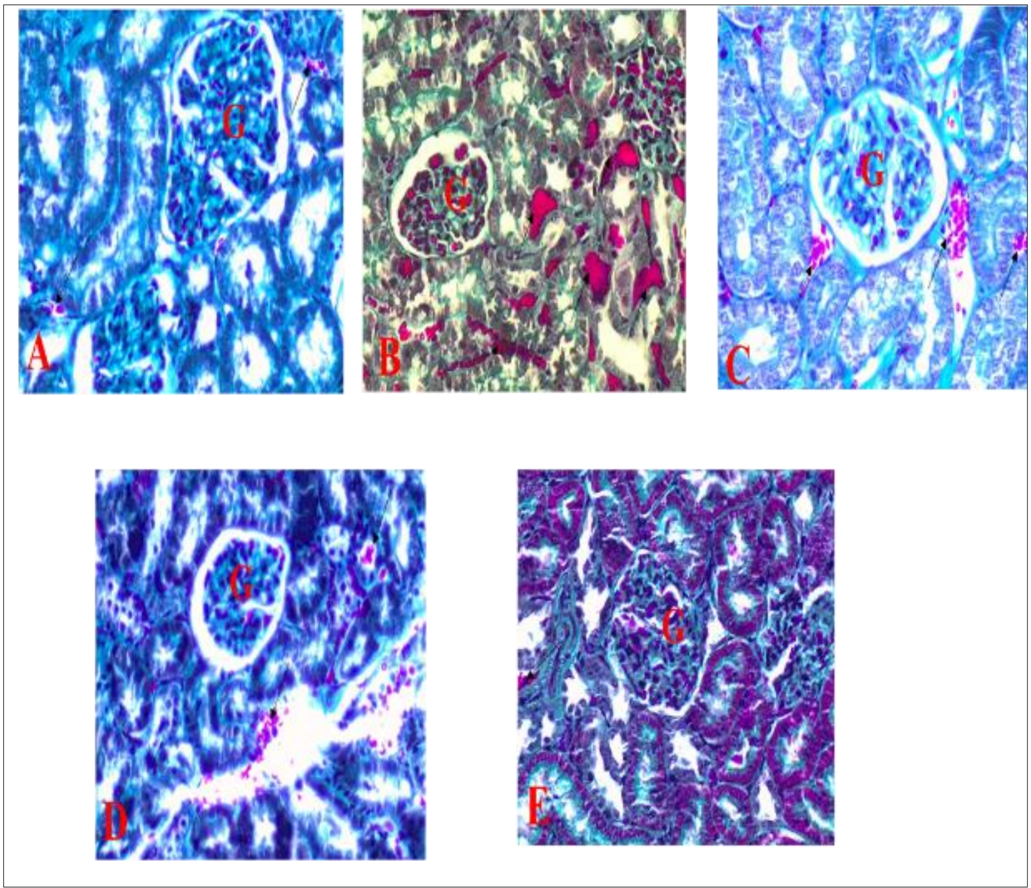

Figure 5 Photomicrograph showing the Kidney at day 28. Masson stain X400. A- Normal control, B – Diabetic, C – Diabetic+Cactus, D – Diabetic+Metformin and E- Cactus only. G - glomerulus, Black arrow – collagen fibre

4. Discussion

In this present study the decline of body weight in the diabetic induced Wistar rats started in week three is attributed to the increase of blood glucose with inhibition of insulin level, decline of tissue proteins, and enhancement of muscle wasting diabetes mellitus is accompanied with increased glycogenolysis, lipolysis, and gluconeogenesis and these biochemical activities result in muscles wasting and loss of tissue protein. The body depends on insulin as a major anabolic hormone. The reduction and insufficiency of insulin caused metabolic disorders of glucose and also lipids and protein. In the diabetes+cactus group there was an increase in the weight compared to those of the untreated diabetic rats group, as demonstrated in figure 1, this is attributed to the high dietary fibers content the cactus that has been reported by [13].

In this study the diabetic induced Wistar demonstrated a marked hyperglycemic state compared to the control and cactus only group. The administration of aqueous extract of cactus demonstrated a significant reduction in the blood glucose levels as illustrated by 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. Several former studies demonstrated the hypoglycemic effect of the cactus plant is attributed to many biochemical properties found in the plant including the presence of insulin-like substances in plants, stimulation of β-cells of the pancreas to produce excess insulin, enhancing insulin action and binding, improving glucose metabolism, presence of high level of fibers which interfere with carbohydrate absorption [14, 15]. Other studies have suggested that the anti-hyperglycemic effect observed could be as a result of phenols and flavonoids that have been reported to be chemical constituents of the cactus plant. Phenols have been reported to improve sensitivity of the tissues to insulin through their free radicals scavenging activity thus lowering the glucose levels in blood [16, 17].

In this study, the relative organ weight in the diabetic was significantly less compared to all the groups as shown in figure 3. this was due to destruction of cell of the kidney in this untreated group, which is attributed to oxidative stress from the hyperglycemic state .

At the end of the fourth week, the photomicrograph [figure 1B] of the diabetic group showed disruption of macula densa and urine space is large.The increase in the levels of blood due to streptozotocin induction increased the production of free radicals, mostly nitric oxide radicals. Accumulation of excessive free oxygen radicals triggers oxidative stress that caused inflammation resulted in the disruption of macula densa [18]. As seen in diabetic patients, there is polyuria which results in hyperfiltration, this leads to a large urine space. In Cactus and metformin treated groups, the photomicrographs [figure 1 C and 1D] showed little disruption in macula densa and a large urine space. This is due to the ability of the extracts to reduce inflammation in the kidney which could either be due to direct or indirect effects through reduced levels of blood glucose and oxidative stress. Flavonoid compounds in the cactus extract worked as an anti-inflammatory agent by suppressing proinflammatory cytokine expression, suppressing the production of reactive oxygen species. Flavonoids has also the ability to inhibit several proinflammatory cytokines including TNF-alpha, IL-1beta, and IL-6 [19]. Flavonoids also inhibits the activation of NF-K due to the high rate of ROS; decreasing NF-K beta activity causes a decrease in TNF alpha expression [20, 21]. The decrease in NF-K beta stimulates cell regeneration in necrotic tubules.

In this study, diabetic group showed a lot of deposition of collagen [figure .2 B]. Cactus and metformin treated groups showed a little deposition of collagen [figure C and D]. this is attributed to the inflammatory process that occurs in diabetes as a result of oxidative stress from the hyperglycemic state [22] which resulted in healing with fibrous tissue which is seen on the photomicrograph.

5. Conclusion

The aqueous cactus extract has the ability to lower blood glucose level and also avert the damage caused by Diabetes mellitus on histoarchitecture of the kidney.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors contributed to the research conception and design, so there is no any conflict of interest to disclose.

Statement of ethical approval

Ethical approval and permission for the study was obtained from Mulungushi University School of Medicine and Health Sciences-Research ethical committee

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