World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

MICROANATOMICAL EFFECT OF AQUEOUS LEAVES EXTRACT OF PSIDIUM GUAJAVA (GUAVA) ON THE PANCREAS OF STREPTOZOTOCIN INDUCED DIABETES MELLITUS IN WISTAR RAT

Uthman Ademola Yusuf[1]*, Christine Banda[1], Abigail Banda[1], Michelo Miyoba[2], Aminat Adejoke Yusuf[3] and Isabel Namfukwe Luambia[1]

[1]Department of Human Anatomy, School of Medicine and Health Sciences, Mulungushi University, Livingstone Campus, Zambia.
[2]Department of Clinical Sciences, School of Medicine and Health Sciences, Mulungushi University, Livingstone Campus, Zambia.,
[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

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

Article Received on 16/01/2024 **Article Revised on 06/02/2024** **Article Accepted on 26/02/2024**

ABSTRACT
Psidium guajava leaves extract has been noted to have antihyperglycemic effects. The present study has been designed to analyze the effects of the guava aqueous leaf extract on the body weight, relative pancreas weight, blood glucose levels and its effects on the histoarchitecture of the pancreas. 30 Wistar rats that weighed between 180-220g were randomly selected into five groups: control, diabetic, diabetic+metformin, diabetic+guava leaves and guava leaves only groups. A low dose of streptozotocin (70 mg/kg) was used to induce diabetes. The rats were treated for a period of four weeks daily with guava leaves aqeous extract and metformin which was the standard drug of comparison. At the end of treatment, the diabetic groups treated with guava leaves extract and metformin body weight, relative pancreatic weight and blood glucouse was statistically significant when compared to the diabetic group ($P<0.05$). The histological analysis revealed normal cytoarchitecture of the pancreatic islet cells of the control and guava leaves only group. The normal histoarchitecture of the islet cells was also maintained in the diabetic group treated with guava leaves. There was some degree of disruption in the histoarchitecture in the islet of diabetic group and diabetic group treated with metformin with slight reduction in the cell with accumulation of elastic fibers mass. The guava leaves extract are capable of lowering the blood glucose level and reverse the damaged caused by diabetes on the pancreas histoarchitecture.

KEYWORDS: Diabetes mellitus, Streptozotocin, Wistar rats, Pancreas, *Psidium guajava.*

INTRODUCTION
Diabetes mellitus (DM) is a group of metabolic diseases, involving inappropriately elevated blood glucose levels. DM has several sub types including type1 and type 2 diabetes mellitus, maturity onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies and steroid use.[1]

The main subtypes of DM are type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), which classically result from defective insulin secretion T1DM and/or action T2DM.[1] T1DM is characterized by autoimmune destruction of the beta cells in the pancreatic islets over months or years which leads to an absolute deficiency of insulin. T1DM is one of the most frequent chronic diseases in children but can occur at any age.[2] Type 2 diabetes mellitus (formerly called non-insulin dependent, or adult onset) results from the body's ineffective use of insulin. This type of diabetes is largely as a result of excess body weight and inadequate physical activity. Gestational diabetes which occurs during pregnancy, is hyperglycemia with blood glucose levels above normal but below values diagnostic of diabetes. Women with gestational diabetes are at increased risk of complications during pregnancy and at delivery. These women and possibly their children are prone to developing type 2 diabetes mellitus.[3]

The classical symptoms of diabetes include polyuria, polydipsia and polyphagia occur commonly in type 1 diabetes mellitus, which has rapid development of

hyperglycemia these symptoms can also be seen in type 2 diabetes mellitus.[3] Other symptoms of diabetes mellitus include weight loss, fatigue, restlessness and body pain.

Regardless of the type of diabetes, complications involve microvascular and macrovascular issues. Microvascular and macrovascular complications are as a result of poorly managed diabetes and include retinopathy, nephropathy, neuropathy and atherosclerotic cardiovascular disease (ASCVD) events.[4]

Treatment and management of diabetes mellitus involves diabetes education with much emphasis on lifestyle modification and glycemic control and the administration of synthetic drugs.

Some plants such as *Psiduim guajava* (guava) leaves have been reported to modedratly reduce blood glucose levels in diabetic patients. *Psidium guajava* (commonly known as guava) a member of the Myrtaceae family, is a common plant which is native to most tropical and subtropical areas worldwide. The plant is not only used as a food but also as a medicinal remedy.[5] Preparation of the leaves has been used in folk medicine in several countries, mainly as an anti-diarrheal remedy and treatment of disorders such a diabetes mellitus.[6] The study will show if the extract can ameliorate the damage caused by diabetes on the Pancreas and provide scientific backing to the traditional use of *Psidium guajava*.

METHODOLOGY

The Guava leaves were 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 begins. The guava leaves were oven dried and pounded. The dry pounded leaves were then sieved to obtain a homogenous powder. The extraction was done using[7] methods.

Animal and Animal management

Thirty presumably healthy adult male Wistar rats (*Rattus norvegicus*) were used to conduct the study. The animals were between 8 to 10 weeks old; body weight (180-220 g). The 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*).

Induction of diabetes

Streptozotocin (STZ) was used to induce diabetes. The rats were weighed, and a baseline glucose level established after the overnight fasting period. The animals were injected intraperitoneally of streptozotocin calculated at a dose of 70 mg/kg body weight and reintroduced to the normal feeding cycle.[8] It took about 72 hours for diabetes to be established in the animals following the administration of streptozotocin, therefore a fasting blood glucose was collected to determine the establishment of diabetes using the tail vein puncture 72 hours after administration of streptozotocin. An Accu-Chek glucometer was used to access blood glucose levels. Animals were considered diabetic with fasting blood glucose levels above 10 mmol/l / ≥ 250 mg/dl.

Experimental design

30 Wistar rats weighing between 180-220 g were randomly selected into five groups (6 per cage); Group A: normal control, Group B: diabetic control, Group C: diabetic +guava D: diabetic +metformin, and Group E: guava leaves only group

Psidium guajava mode of administration

The dose of the aqueous extract of Guava leaves used in the study will be adopted from the report of.[9] Guava leaves extract was dissolved in physiological saline daily and was administered orally with use of oro-gastric cannula to Group C rats (n=6) at 200 mg/kg bw (at 9.00 – 10.00 a.m. each day) for a maximum period of four weeks, Group D (n=6) at 200 mg/kg bw of Metformin, Group E rats (n=6) were administered 200mg/kg bw of Guava leaves extracts. Group A rats (n=6) received neither STZ nor Guava leaves extract.

Measurement of blood glucose

The blood glucose was evaluated in overnight fasted rats at 9:00 – 10:00 hours using Glucose oxidase method of one touch ultra 2 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 one weeks (acclimatisation period) before the induction of Diabetes and for four weeks of treatment.[10]

Measurement of body weight (g)

Body weight (g) of the rats was recorded for one weeks (acclimatisation period) before 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).[11]

The Relative Organ Weight (%)

The relative organ weight of the rat was assessed as the ratio of respective weight of the pancreas and the terminal body weight of the same rat, the unit was recorded as percentage (%) using sensitive weighing balance (SonyF3G brand).[12]

Histological process

At the end of the study, the animals were sacrificed by euthanasia. They were laid supine on the dissecting board and pinned through the fore and hind paws. The animals were dissected with Scalpel and surgical blade and the pancreas was carefully removed and weighed. The tissue for histological studies was fixed in freshly prepared formol saline for 72 hours and processed for routine histological examinations stained with

Haematoxylin and Eosin (H&E) to observe changes in the cellular morphology. Special stains such as Von Giesson for elastic fibers and Gomori for Beta cells are also demonstrated.

Data collection procedure

Data collection was obtained by measuring the body weight and blood glucose levels before and after drug administration then recorded in a data entry sheet for a period of four weeks. After sacrifice of the rats, relative organ weight was measured and histological analysis of pancreas was carried out.

Statistical analysis plan

Data was presented as mean ± standard error of the mean (mean±SEM); analysed using one-way ANOVA and all graphs were drawn using excel. P values less than 0.05 ($p<0.05$) was taken statistically significant.

RESULTS

Average body weight on weekly basis

Figure 1 shows the average body weight of the rats on a weekly basis. In the week of acclimatization (week -1), there was no significant change in body weight among the groups. After induction (week 0), a slight increase in the bodyweight across all groups were noted however the increase were not significant ($p >0.05$). By week three of treatment there was a reduction in the bodyweight of rats in diabetic group ($p<0.05$) compared to those in the control, diabetic +guava leaves and diabetic +metformin. The diabetic group's weight continued to reduce until the end of treatment. There was no significant change ($p>0.05$) in the control and guava leaves only groups when compared.

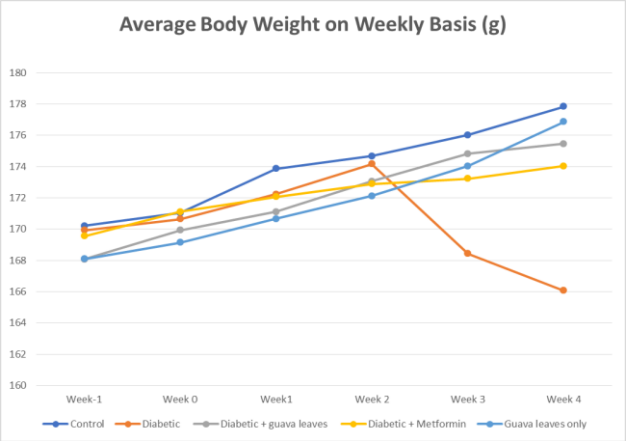

Figure 1: Average body weight (g) on weekly basis. Data expressed as mean±SEM ($p<0.05$).

Blood Glucose on Weekly Basis (mg/dl)

Figure 2 shows blood glucose on a weekly basis in mg/dl. After induction, increased levels of blood glucose were noticed in diabetic group, diabetic +metformin and diabetic + guava leaves. In week 1 and 2 there was significant ($p <0.05$) decrease in the blood glucose in the diabetic + Metformin and diabetic +guava leaves as compared to the diabetic group. However, by the third week, the blood glucose reduced more significantly ($p<0.05$) in the diabetic +guava leaves compared to the diabetic +metformin. There was no significant ($p>0.05$) change in blood glucose in the control and guava leaves only group. The blood glucose in the diabetic group continued to increase. By the end of treatment (28 days), the animals in the diabetic +guava leaves and diabetic +metformin groups returned blood glucose levels to normoglycemic when compared to control group.

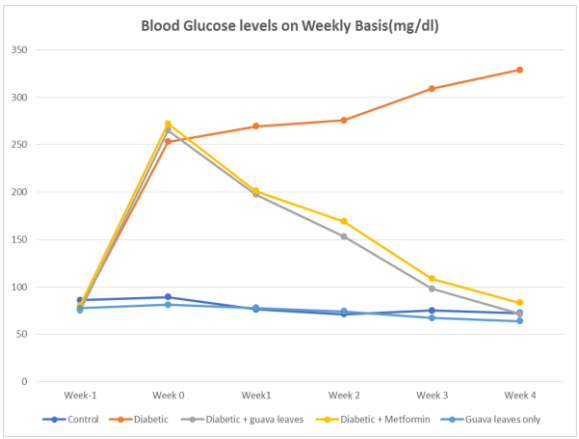

Figure 2: Shows the blood glucose (mg/dl)on a weekly basis . Data expressed as mean±SEM ($p<0.05$).

Relative pancreatic weight

Figure 3 shows a graph of the relative pancreatic weight. The relative pancreatic weight in the control group was higher compared to the other groups. However, there was no significant ($p>0.05$) difference in the relative pancreatic weight of the control group and guava leaves only group. The diabetic group relative pancreatic weight was the lowest when compared to other groups it was statistically significant ($p<0.05$).

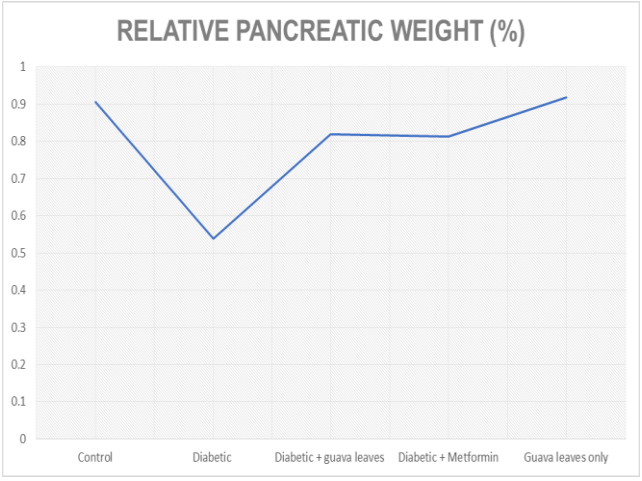

Figure 3: Shows the relative pancreatic weight. Data expressed as mean±SEM ($p<0.05$).

Histological findings of the pancreas

The normal control and Guava only groups showed normal cytoarchitecture of cells in the Pancreatic islets (Plate: 1A, 2A, 3A and 1E, 2E, 3E); there were disruption of the cytoarchitecture, reduced cell mass in the Pancreatic Islet and accumulation of elastic fibers of the diabetic groups (Plate: 1B, 2B, 3B). The normal cytoarchitecture was maintained in the pancreatic islet of Diabetic+Guava and Diabetic+metformin groups (Plate 1C and D 2 C and D, 3 C and D). There was also accumulation in β cell mass in this group (Plate: 1C and 1 D). There was some degree of disruption in the cytoarchitecture in the islet of diabetic group and Diabetic+Metformin group with slight reduction in the cell mass (Plate:3B, and 3D). Elastic fibers were extensively accumulated with high intensity in the diabetic group and Diabetic+Metformin groups (Plate: 3B and 3D).

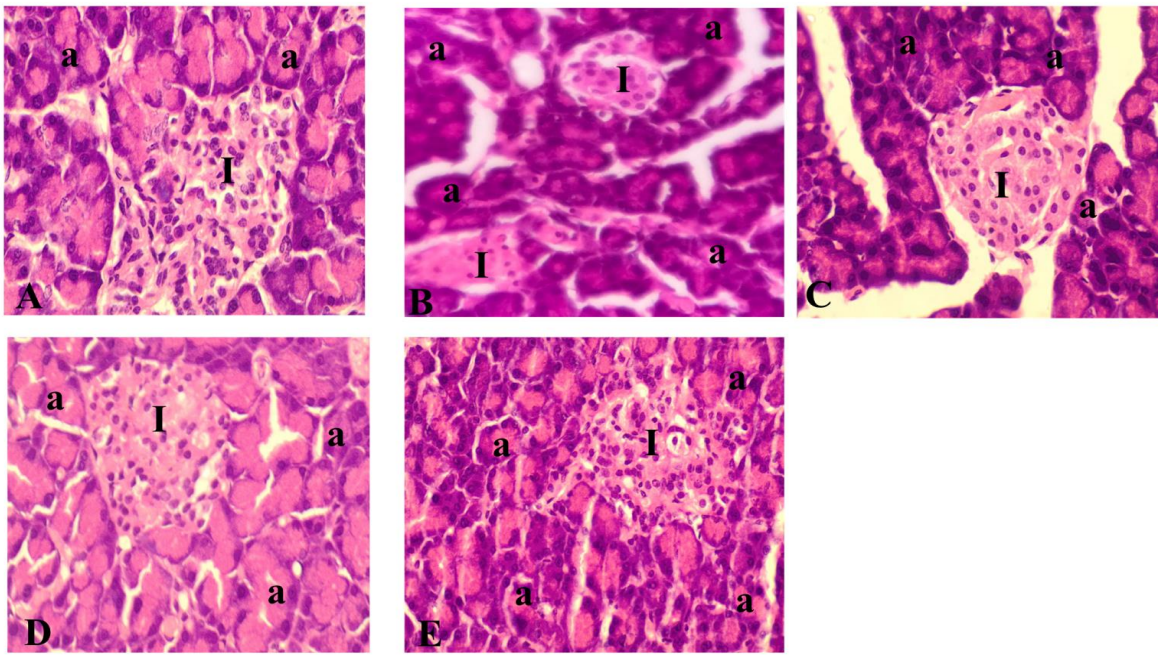

Plate 1: Photomicrograph showing the pancreatic islet at week four. H&E X400. A- Normal control, B – Diabetic, C – Diabetic+Guava, D – Diabetic+Metformin and E- Guava only. I- Pancreatic islet, a-Acini.

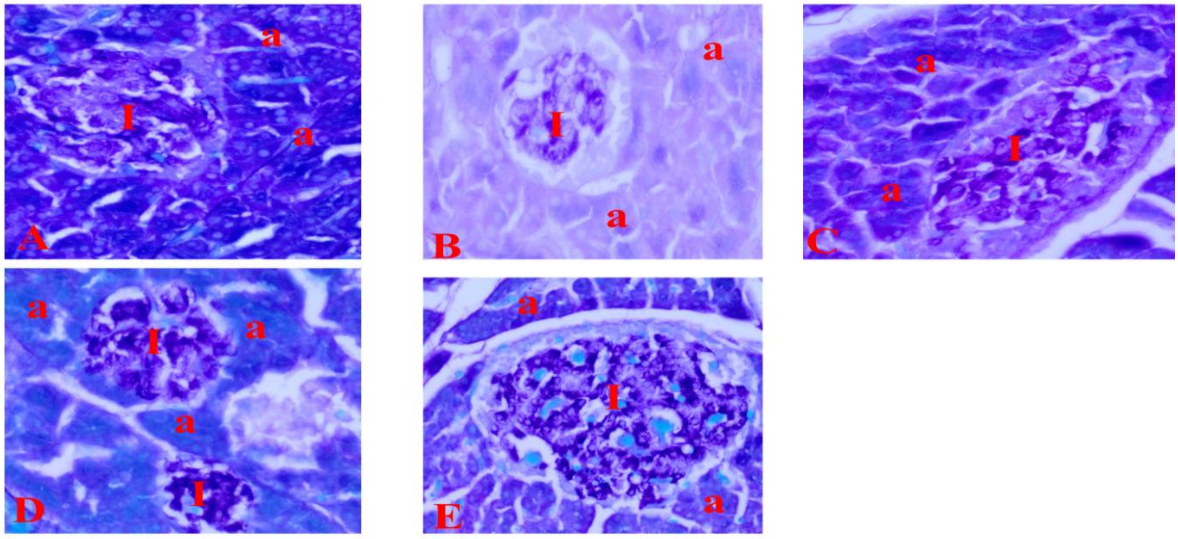

Plate 2: Photomicrograph showing the pancreatic islet at week four. Gomori X400. A- Normal control, B – Diabetic, C – Diabetic+Guava, D – Diabetic+Metformin and E- Guava only. I- Pancreatic islet, a-Acini.

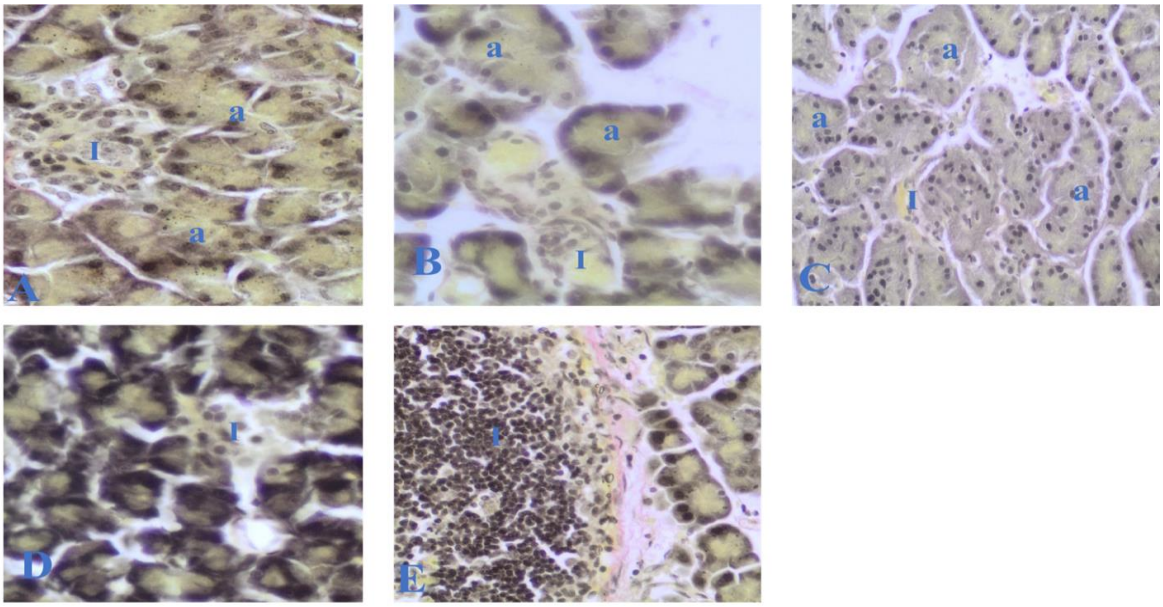

Plate 3: Photomicrograph showing the pancreatic islet at week four. Von Giesson X400. A- Normal control, B – Diabetic, C – Diabetic+Guava, D – Diabetic+Metformin and E- Guava only. I- Pancreatic islet, a-Acini.

DISCUSSION

Diabetes mellitus is a chronic metabolic condition that is characterized by inappropriately raise of blood glucose levels**,** to treat diabetes mellitus lifestyle modification and potent anti-diabetic drugs have been used to manage and treat the condition. In this study, a rat model was established with Diabetes mellitus by inducing with 70mg/kg body weight of streptozotocin with aim of identifying the effects of *psiduim guajava* leaves extract on the body weight, blood glucose, relative pancreatic weight as well as the histological effects of the extract on the pancreas.

After the second, it was noted that the body weight of the diabetic group rats began to decrease compared to the other groups, this was due to the increase of glucose via gluconeogenesis by lipolysis, decrease in tissue proteins and increase in muscle wasting in the diabetic rats which was in agreement with.[13] There was body weight decreased in the diabetic+guava leaves and diabetic + metformin it was not significant when compared to control and guava leaves only groups. This effect was attributed to the improvement of glycemic control and insulin levels, increase in insulin levels saves the required glucose as a source of energy for cells to get their caloric needs and the process of proteolysis and lipolysis are reduced. This result is in agreement with.[13]

In week 1 and 2 it was observed that there was an increase in the blood glucose levels of the diabetic group, the diabetic+guava leaves and diabetic+ metformin when compared to the control and guava leaves only groups.

By the end of treatment, it was observed that the diabetic+guava leaves group blood glucose levels returned to normoglycemic. This finding shows that *psiduim guajava* leaves extract has anti-hyperglycemic effects in diabetes as in a similar study reported by[9] Several studies have been performed to explain the effects of the anti-diabetic compounds present in the guava leaves extract. According to[14] the anti-hyperglycemic compounds contained in the guava leaves extract in particular quercetin was found to promote glucose uptake in hepatocytes and alleviate hyperglycemia in diabetes. It was also found that the polysaccharides and flavonoid compounds delay absorption of glucose in the small intestines by inhibiting a-glucosidase and a-amylase ultimately reducing the blood glucose levels.[15,16]

The study showed that guava leaves extract preserved the relative pancreatic weight in diabetic+guava leaves rats when compared to the control group. This was due to the numerous bioactive polyphenolic compounds, such as flavonoids, ferulic, caffeic and gallic acid apart from the polyphenolic ccompounds it also contains a number of bioactive polysaccharides, proteins, lipids, vitamins and minerals which exhibit strong antioxidant and immunostimulant activities.[7,16]

By the end of week four of treatment, the histological analysis of the pancreas was done using three stains (H&E, Gomori and Von Giesson), the analysis revealed normal cytoarchitecture of the pancreatic islet cells was maintained in the control and guava leaves only groups (Plate: 1A, 2A, 3A and 1E, 2E, 3E). This finding demonstrated that guava leaves extract had no pathological effects on the pancreas of the guava leaves only group. The H&E, Gomori and Von Giesson stains in the diabetic group (Plate: 1B, 2B, 3B); shows that there were disruption of the cytoarchitecture, reduced cell mass in the Pancreatic Islet and accumulation of elastic fibers. This was due to the excessive production of reactive oxygen species that induced cytotoxicity in beta cells of the pancreas by streptozotocin17] this finding was similar to that of a study done by[9,19] The H&E, Gomori and Von Giesson stains also revealed normal cytoarchitecture of the pancreatic islet of the diabetic+guava leaves and Diabetic+metformin (Plate 1C and D, 2 C and D, 3 C and D) these groups also showed accumulation in β cell mass (Plate: 1C and 1 D) this was due to the antioxidants, flavonoid and phenol compounds present in the guava leaves extract which prevented further destruction of the cells and enhanced proliferation of the beta cells.[7] and the protection from lipotoxicity or glucotoxicity by metformin though the mechanism of action by which this effect is made is still unknown.[18,19] The Von Giesson stain showed some degree of disruption in the cytoarchitecture of the islet with slight reduction in the cell mass of the diabetic group and diabetic+metformin group (Plate: 3B, and 3D). Elastic fibers were extensively accumulated with high intensity in the diabetic group and diabetic group treated with metformin (Plate: 3B and 3D) this could be due to oxidative stress and release of reactive oxygen species and super oxide caused by the hyperglycemia.[17] The accumulation of the elastin fibers signified that inflammatory processes and degradation of the tissue was happening due to the oxidative stress and hyperglycemia.[20]

CONCLUSION

This result showed that guava leaves extract exhibited an anti-hyperglycemic effect and the capacity to reversed the damage commenced by hyperglycemia on the Pancreas of male Wistar rats.

REFERENCES

1. Sapra A and Bhandari P. Diabetes Mellitus. In: Statpearls [internet]. Treasure Island (FL): StatPearls Publishing, 2022.
2. Lucier J and Weinstock RS. Diabetes Mellitus Type 1. In: Statpearls [internet]. Treasure Island (FL): StatPearls Publishing, 2021.
3. World Health Organization, 2021. Diabetes. Retrived from https://www.who.int/news-room/fact-sheets/detail/diabetes. Accessed on 23/12/2021
4. Goyal R and Ishwarlal J, 2021. Diabetes Mellitus Type 2. In: StatPearls[internet]. Treasure Island (FL):StatPearls Publishing
5. Uthman Ademola Yusuf, Mwewa Masamba, Betty Banda, Sam Beza Phiri, Michelo Miyoba, Aminat Adejoke Yusuf, John Mulemena, Kingsley M Kamvuma, Olubukola Iyanuoluwa Ademola Maintenance of Renal integrity of Diabetic Wistar Rats treated with aqueous extract of *Psidium guajava* MUMJ, 2023; 4(1): 162-170.
6. Diaz-de cerio E, Verardo V, Gomez-Caravaca A, Fernandez-Gutierrez A, and Segura-Carretero A Health effects of psidium guajava L. leaves: An overview of the last decade. *International journal of molecular sciences*, 2017; 18(4): 897. doi: 10.3390/ijms18040897
7. Kumar M., Tomar M, Amarowicz R, Saurabh V, Sneha Nair M, Maheshwari C, Sasi M, Prajapati U, Hasan M, Singh S, Changan S, Prajapat R.K, Berwal M.K, and Satankar V. Guava (Psidium guajava L.)Leaves: Nutritional compostion, Phytochemical Profile, and Health-Promoting Bioactivies. *Foods*, 2021; 10(4): 752. doi: 10.3390/foods10040752.
8. Yusuf U.A., Sijumbila G., Mesole B., Mulemena J.A., Kamvuma K., Eweoya O.O., Adenowo K.T. Histological study on the effects of aqueous extracts of citron leaf on pancreas of hyperglycemic Wistar rats. *Biol sci pharma res*, 2019; 7(5): 82-90.
9. Jayachandran M.,Vinayagam R., and Xu B Guava leaves extract ameliorates STZ induced diabetes mellitus via activation of PI3K/AKT signaling in skeletal muscle of rats. *Mol Biol Rep*, 2020; 47(4): 2793-2799.
10. Uthman Ademola Yusuf, Abigail Banda, Christine Banda, Sam Beza Phiri, Michelo Miyoba, Aminat Adejoke Yusuf, John Mulemena, Kingsley M

Kamvuma Olubukola Iyanuoluwa Ademola. *Psidium guajava* leaves extract and Antidiabetic: Cardioprotective effects in diabetic male Wistar Rats. WJPPS, 2023; 12(6): 59-73.
11. Yusuf U.A., Adeeyo O.A., Adegoke A.A., Mesole B.S., and Okeniran O.S. Neurobehavioural Study on the Effect of Aqueous Extract of Citrus Medica Leaf on Prefrontal Cortex of Hyperglycemia Wistar rats. *J Mol Histol Med Physiol*, 2018; 3: 123.
12. Uthman Ademola Yusuf, Wana Iputu, Musonda Kambele, Mpeza Kalowa, Michelo Miyoba,Isabel Namfukwe Luambia, Precious Simushi, Aminat Adejoke Yusuf, Memory Ngosa. A Histological Study on the Effect of Aqueous Extract of Cactus on Frontal Cortex of Diabetes Mellitus Wistar Rat. European Journal of Pharmaceutical Research, 2023; 3(2): 5-11.
13. Shadia A.R., Yasser A.K., Gamal A.H., and Omnia A.M Effect of *Psidium guajava* leaf extract, glibenclamide and their combination on rat model of diabetes induced by streptozotocin. *The Egyptian Journal of Hospital Medicine*, 2018; 72(6): 4610-4619.
14. Chu S., Zhang F., Wang H., Xie L., Chen Z., Zeng W., Zhou Z., and Hu F. Aqeous Extract of Guava (*Psiduim guajava* L.) Leaf Ameliorates Hyperglycemia by Promoting Hepatic Glycogenic Synthesis and Modulating Gut Microbiota. *Front Pharnacol, 2022;* 13: 907702. doi: 10.3389/fphar.2022.907702
15. Zhang Z., Kong F., Ni H., Mo Z., Wan J.B., Hua D., Yan C. Structural Characterization, alpha glucosidase Inhibitory and DPPH Scavenging Activities of Polysaccharides from Guava. *Carbohydr. Polym*, 2016; 144: 106-114. doi:10.1016/j.carbpol.2016.02.030
16. Uthman Ademola Yusuf, Musonda Kambele, Wana Iputu, Mpeza Kalowa, Michelo Miyoba, Isabel Namfukwe Luambia Luambi, Precious Simushi and Aminat Adejoke Yusuf Histological investigation of aqueous extract of cactus on the heart of diabetic Wistar rats. GSC Biological and Pharmaceutical Sciences, 2023; 22(01): 134–142.
17. Adeeyo OA., Dare B.J., Yusuf U.A., Okeninan O.S., Ebiwonjumi A.S., Ojo W.A., Insulin Profiling and Histo-pancreatic architecture in STZ-induced Diabetes Mellitus Rats Model Treated with Mistletoe and Drum stick Aqueous Leaf Extracts. European Journal of Pharmaceutical and Medical Reasearch, 2018; 4(2): 114-128.
18. Yang X., Xu Z., Zhang C., Cai Z., and Zhang J Metformin, beyond an insulin sensitizer, targeting heart and pancreatic β cells. *Molecular Basis of Disease, 2017;* 1863(8): 1984-1990.
19. Uthman Ademola Yusuf, Mpeza Kalowa, Musonda Kambele, Wana Iputu, Memory Ngosa, Isabel Namfukwe Luambia Luambi, Precious Simushi and Aminat Adejoke Yusuf Micro anatomical effects of aqueous cactus extract in the lungs of a diabetic Wistar rat. World Journal of Advanced Research and Reviews, 2023; 17(01): 410–416
20. Heinz A Elastic Fibers During Aging of Disease. *Ageing Research Review*, 2021; 66: 101255. https://doi.org/10.1016/j.arr.2021.101255