Original Research Article

# CHANGES IN BLOOD GLUCOSE AND GLYCATED HAEMOGLOBIN LEVELS IN ALLOXAN-INDUCED FEMALE WISTAR RAT TREATED WITH *AZANZA GARCKEANA* FRUIT EXTRACT




## ABSTRACT

**Background: Insulin-related defects, whether in secretion or action, lead to a conglomerate of metabolic disorders with elevated glycemic index known as diabetes. Due to high cost of synthetic drugs, scientific research is being done to explore the use of herbal extracts to ameliorate this disease. Azanza garckeana fruits are edible and are most useful as medicinal agents. This research explored the effect of this plant on blood glucose and glycated haemoglobin levels in alloxan induced diabetic female wistar rats.**

**Method: Experimental Diabetes mellitus was induced with a single intraperitoneal injection of alloxan (150 mg/kg) in all test groups except the normal control. The study examined rats that were considered diabetic and had fasting blood glucose levels 250 mg/dL. 1. Group 1 rats without diabetes were given distilled water as a standard control method. group 2 was alloxan only(Diabetic control) and group 3-5 was treated with the herbal extract 250mg, 500mg and1000mg/kg respectively, while group 6 was given a standard antidiabetic medication (glibenclamide at 10 mg/kg body weight). During day 1, 3, 7, 14, 21 and 28 days of treatment, the blood glucose levels were measured, whereas glycated hemoglobin was measured on the 28$^{th}$ day.**

**Results: Blood glucose levels in alloxan-induced rats were significantly raised from 4.34 mmol/L on day 1 of treatment to 18.26 mmmol/1. The use of herbal extract, which was more effective than synthetic glibenclamide, resulted in a marked decrease in blood glucose levels from day 14 to 28. The impact on blood glucose levels may be due to the combined efforts of the five most prevalent phytochemicals, including octadecanic acid and ethyl ester, which have a higher affinity for binding to sulfonyl receptors than glibenclamide. The glycosylated hemoglobin levels in group 2 were significantly elevated when treated with alloxan, but decreased dramatically when given the herbal extract of 250mg/kg, 500mg/kg, and 1000mg/kg, as well as when exposed to synthetic drugs.**

**Conclusion: Blood glucose levels in rats were found to be significantly higher after alloxan was administered but the treatments significantly decreased blood glucose levels, with the herbal extract proving more effective than the synthetic drug (glibenclamide).**


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## 1. INTRODUCTION

Diabetes is a conglomerate of metabolic disorders associated with high glycemic index due to defects in insulin (or insul-hormon) secretion, action or both. We have Type 1 and Type 2 diabetes mellitus; The Type 1 category is also known as Insulin dependent diabetes mellitus (IDDM) because it is caused by lack of insulin by the beta cells of langerhans of the pancreas. Type 2 Diabetes also called Non –IDDM is due by dampened sensitivity of target cells to the regulatory insulin hormone substance (Ozougwo *et al.,* 2013).

According to WHO, the global incidence level of the disorder amongst adults in 2010 was 285 million (6.4%) and this figure is predicted to rise to around 439 million (7.7%) by 2030 (Shaw *et al.,* 2010).

Chronic hyperglycemia along with deviations in protein, lipid, and carbohydrate metabolism brought on by deficits in either insulin hormone action or secretion, or both, is the hallmark of Diabetes Mellitus. It can cause prolonged harm and organ failure and malfunction. It can also result in continuous thirst stimualtion, polyuria, blurred eyesight, and weight loss, etc (Butler & Misselbrook, 2020). In its most extreme forms, ketoacidosis, also known as a non-ketotic hyperosmolar state, can occur. This condition can cause stupor, coma, and, in the event that treatment is not successful, death. Frequently, there may be no symptoms at all or only mild ones, which means that hyperglycemia up to huge extent may result in functional shifts may exist for a long time prior to diagnostics.

When someone presents with severe symptoms and extensive hyperglycemia, different parameters are expected to be explored for reliable diagnostic probe as against cases with obvious symptoms glucose levels with mild elevations beyond the diagnostic cut-off level (Raju & Raju, 2010). In the case of marked sugar level, found during a critical contractile stage, circulatory, or other stressful scenarios. This approach is only a brief one and is taken as a preliminary approach in profiling the condition.The investigation or diagnosis of the condition in one with no obvious indications in just a plasma check may be illusive. An

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additional follow up of fasting level of glycemic value could be very helpful in ascertaining the actual diabetic profile non-symptomatic individuals.

It is normally advised to continue surveillance with retests on a regular basis until the diagnosis is apparent if such samples are unable to confirm the diagnosis of diabete mell. When choosing a diagnostic or treatment plan in these situations, the clinician 4 should also consider other criteria such age, obesity, family history, ethnicity, and concurrent illnesses. For a long time, a simpler method to diagnose diabetes has been sought after—an alternative to blood glucose measurement or the OGTT. It was believed that glycated haemoglobin, which represents average glycaemia over a few weeks, might offer such a test.

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In most scenarios, the likelihood of controlling the carbohydrate intake is vital to the individual, whether the goal is to avoid or control diabetes, lose weight, or simply preserve a healthy weight and lifestyle. It is possible that the individual have already cut back on high-glycemic (or simple) carbohydrates in favor of whole grains, fruits, and vegetables, which are higher in nutrients. Well done if that is the case. It can also be beneficial to pay more consideration to the Glycemic load of the meals the individual eat.

Carbohydrate-containing foods are ranked on the Glycemic Index (GI) which measures how much they can increase blood sugar. It is an additional method of basing dietary decisions on the many physiological effects of carbohydrates. The system operates on a 0–100 scale, where 0 corresponds to a food that contains no carbs (like butter) and 100 to pure glucose. It was first created to assist those who were pre-diabetic or had diabetes in choosing healthier foods. While not every item has been investigated and given a GI, the list is long and undoubtedly includes most of the meals consumed in industrialized countries.

The body converts sugar and starches from foods high in carbs into glucose, which is the main energy source for cells. The rate at which different kinds of carbohydrates break down and reach the bloodstream determines how they function. Likened to an equivalent portion of carbohydrates from whole grain bread and fiber-rich broccoli, the sugar (carbohydrates) in a can of soda will break down more quickly. This implies that drinking soda will raise the blood sugar levels, which will then quickly fall to what are typical levels in someone who is fit. However, over time, insulin resistance and other health issues may result from those spikes and troughs.

Foods are ranked by their Glycemic Index values (low, medium, and high). The effect on blood sugar elevation will be smaller the lower the value. Low-glycemic foods, such as legumes, most vegetables, dairy products, and nuts, do not quickly raise or lower blood sugar levels. However, meals high in glycemic index (GI), like most processed foods, baked goods, fast food, and white bread, can cause the blood sugar to spike quickly, which can immediately cause it to drop again and leaving the individual feeling famished and lethargic. Whole grain breads, many fruits, and starchy vegetables are among the foods having a medium GI rating. Foods with more fat and fiber content typically have lower glycemic index (GI) values. Here the individual may look up the GI values of different foods.

However, a food's GI does not always provide all the information. It disregards additional crucial nutritional factors including the amount of calories, fat, fiber, protein, and vitamins and minerals that different foods may have. Many foods with a low GI may be deficient in nutrients, whereas those with a medium or high GI may offer a wealth of additional nutritional advantages that are essential for a balanced diet. For this reason, it is crucial to take into account a food's glycemic load. A food's ability to provide a specific amount of glucose as well as how soon it will convert to glucose and enter the bloodstream is measured by its Glycemic load. The grams of carbohydrates in a food are multiplied by the GI index to determine the Glycemic load, which is then divided by one hundred. Meals with a Glycemic load of twenty or more are classified as high Glycemic , those with a Glycemic load of eleven to twenty as medium, and meals with a Glycemic load of less than ten as low. Watermelon is the food that nutritionists use the most frequently to highlight the significance of the Glycemic load. Watermelon has a high GI of eithy, but only around seven grams of carbohydrates per serving, so its Glycemic load is just about 5.6, making it a low GL meal.

Gaining knowledge regarding the glycemic load and index of different foods will undoubtedly assist the individual in making healthier choices. It is probably not necessary for most people to figure out the nutritional value of every food they eat. Nutrient-dense foods predominate in a balanced, diverse diet, but minimal amounts of high GI foods—which can seriously disrupt blood sugar levels—are included. Discussing how a diet rich in lower GI and Glycemic Load items can help the individual better handle the overall wellness with the doctor or a licensed nutritionist is a good idea if the individual're diagnosed with

diabetes, are worried about blood sugar swings or insulin resistance. Additionally, the individual should always consult the doctor before making any marked dietary changes.

**Insulin Hormone**

It is a polypeptide, made of 51 amino acids. It is essential for cell growth, metabolism, and glucose homeostasis. Insulin hormone is primarily secreted by beta cell in the pancreatic islets of Langerhans, although small amounts have also been detected in some central nervous system neurones. Glucagon and it could synergistically control the glycemic concentrations; with its anabolic technique, insulin hormone does the work of effective control and the other hand glucagon acting catabolically, homeostatically make available glycemic value for peripheral purposes. This attribute of insulin ultimately result in weight gain in the subject. The synthesis and levels of the hormone exerts an essential effect in the start and continuity of several prolong conditions because it modulates a extensive collecction of functional activities.

Circulating glucose levels control insul-hormon synthesis and release. Insul-hormon secretion cannot occur at glucose concentrations below 5 mmol; instead, insulin biosynthesis is stimulated by variations in glucose levels between 2-4mmol.

When food is consumed, beta cell insulin production increases and α cell glucagon production decreases, resulting in increased glucose metabolism and normal serum glucose levels. After secretion, circulating insul-hormon stimulates the liver, skeletal muscle cells, and adipocytes to store glucose as glycogen, which brings the blood glucose level back to normal. Insulin hormone not only promotes the uptake of glucose but also initiates lipogenesis and protein synthesis in skeletal muscle via an efficient pathway.

Phosphates from ATP are transferred to special residues intracellularly by the insul-hormon receptors that are found in the plasma membrane through an enzymatic process. Insul-hormon binds to the alpha subunits, causing the beta branches to attach phosphate and activate the receptor's catalytic function. Additionally, the activated receptor phosphorylates a number of intracellular proteins that control insulin metabolism, cell division-related gene expression, and cell growth.

***Physiological Roles of Insulin***

It basically determines the body's energy input via conserving the proper balance of micronutrients. Moving intracellular glucose to insulin-contolled cells/tissues, including but not limited to liver, muscle, and adipose tissue, is a critical function of insulin . After being released, the hormone then arrives all other organs with its receptors via the bloodstream. Insulin aids in the movement of blood glucose into hepatocytes in the liver, where it is subsequently transformed into fatty acids, TG, and glycogen. Conversely, insulin decreases the amount of glucose produced by gluconeogenesis, glycogenolysis, and net glucose production. Adipose tissue and skeletal muscle absorb glucose more quickly than other tissues, causing elevated blood glucose levels to drop to baseline through glycogenesis. It has been proposed that insul-hormon affects the liver both directly and indirectly, though the exact process by which it controls hepatic function is still unknown. It has been shown in either of in vitro and in vivo experimentations that it can directly bind with hepatic insul-hormon receptors, activating insul-hormon signalling pathways in the liver in the process. Conversely, the prevention of fat lipolysis, the decrease in pancreatic glucagon flow, and the overall influence of hypothalamic insul-hormon signalling control indirect insulin action, which in turn influences hepatic glucose production. Despite data showing both straight and non-straight effects of insulin on the hepatic tissue, although it is insinuated that most of the modulations of hepatic tissues is rather indirect. The hormone makes it easier for the skeletal muscles to absorb amino acids and glucose from the blood. After that, the amino acids are used for the synthesis of functional proteins, and glucose is primarily used in glycolysis to create ATP, which is the body's energy source. Moreover, glucose can be transformed into glycogen, which is primarily conserved as energy for low-energy states. Insulin promotes the uptake of fatty acids by adipose tissue, where they are subsequently transformed into TG and utilised as long-term energy reserves. It's crucial to remember that every action or process in the figure that insul-hormon controls is reversible. Insul-hormon usually causes these processes to be irreversible once it does so.

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### 1.1. *Azanza garckeana* AGF

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The plant is an edible fruit in Northern Nigeria has been indicated to have fertility potentials and is being used by the locals to treat infertility amongst other conditions (Dikko *et al.,* 2016; Ochokwu *et al.,* 2015). It falls into the Malvaceae family and also called Goron-Tula in Nigeria, Morojwa in Botswana and in English it is known as Snot Apple and many other names depicting its attributes and applicatons at different quarters

(Abba *et al.,* 2018). It can be found in Nigeria, Congo, Sudan, etc (Mojeremane & Tshweyane, 2004; Orwa *et al.,* 2004; Ochokwu *et al.,* 2005). In Nigeria, it is predominant and habitually cultivated by the people of Tula, Kaltungo Local Government Area of Gombe State and their neighbours (Michael & Tengas, 2015: Abba *et al.,* 2018).

Goron Tula as commonly known in Nigeria is a deciduous small tree or shrub that can grow up to 3-15m tall depending on environmental conditions (Abba *et. al.,* 2015). The diameter of the stem at breast height can be up to twenty five centimeter (Orwa *et al.,* 2009; Maroyi, 2017). The seed hemisperenis up to 10 mm long and 7 mm thick with brownish and wooly floss. (Michael *et al.,* 2015). As shown in figure 1, the plant has finely ovoid leaves (that is eight by twelve centimeters on a streched stalk) (Ochokwu *et al*., 2015; Maroyi, 2017). The flowers get to 6cm in length; the colours are centrally deemed red and yellow purple across other portions

*Azanza garckeana* has so much dietary and medicinal uses. The fruits are edible and are most useful.they are eaten while slightly green or ripe or dried and reconstituited later (Mojeremane &Tshweneyare 2004). The pulp is chewed like a chewing gum and produces a sweet glutinous taste (Jacob *et al.,* 2016). They can be soiaked in a small amount of water to form jelly or boiled and made into porridge. The leaves are also eaten as vegetable (Ochokwu *et al.,* 2015). The various portions of the plant is known to possess medicinal attributes and are used to manage conditions in areas where the plant is found.

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## 2. METHODS

### 2.1. Experimental design

The present study was a laboratory-based experimental study using Wistar rat models. The work was carried out in the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt.

### 2.2. Preparation of Azanza garckeana Extract

Fruits of *Azanza garckeana* were collected from Tula Town, Kaltungo Local Government Area, Gombe State, Nigeria, where the plant is widely cultivated. The plant material was identified and authenticated by Dr Ekeke Chemezie, a plant taxonomist at the University of Port Harcourt Herbarium, Nigeria. A voucher specimen (UPH/P/414) was prepared and deposited at the Herbarium for reference. The fruits were separated into pulp and seeds. The pulps were air-dried at ambient temperature (approximately 37°C) and subsequently ground into a fine powder using an electric grinder. The powdered material was macerated

in a hydroethanolic solvent (ethanol–water, 70:30 v/v) with intermittent stirring to ensure adequate extraction. The mixture was then filtered and concentrated using a rotary evaporator maintained at 60°C. The resulting extract was stored at 4 °C until further use (Ahmed et al., 2016).

## 2.3. Experimental Animals

Thirty (30) adult female Wistar rats (*Rattus norvegicus*) weighing 150-200 grams were used for the study. The Wistar rats were obtained from the Department of Physiology Animal House, University of Port Harcourt and housed under standard laboratory conditions (temperature 25 ± 2 °C, and 12-hour light/dark cycle). The rats were allowed free access to feed and clean drinking water *ad libitum*. All rats were allowed to acclimatise for 14 days before the commencement of the experiment.

### 2.3.1. Induction of Experimental Diabetes

Experimental Diabetes mellitus was induced in all test groups, except the normal control, by a single intraperitoneal injection of freshly alloxan monohydrate at a dose of 150 mg/kg body weight, dissolved in 0.9% normal saline. To prevent initial hypoglycaemic shock, rats were provided with 5% glucose solution for 24 hours post-alloxan administration. After 72 hours, fasting blood glucose levels were measured using a glucometer via tail vein puncture. Rats with fasting blood glucose levels ≥250 mg/dL were considered diabetic and included in the study.

### 2.4. Experimental Grouping and Treatment Protocol

A total of thirty (30) rats were randomly assigned into five experimental groups (n = 6 per group) as follows:

A total of thirty (30) rats were randomly as signed into five experimental groups (n = 6 per group) as follows:

1. Group 1 (Normal control): Non-diabetic rats administered distilled water.
2. Group 2 (Diabetic control): Alloxan-induced diabetic rats were administered distilled water.
3. Group 3: Diabetic rats treated with Azanza garckeana extract at 250 mg/kg body weight.
4. Group 4: Diabetic rats treated with Azanza garckeana extract at 500 mg/kg body weight.
5. Group 5: Diabetic rats treated with Azanza garckeana extract at 1000 mg/kg body weight.
6. Group 6: Diabetic rats treated with a standard antidiabetic drug (glibenclamide at 10 mg/kg body weight).

All treatments were administered orally once daily using an oral gavage for a period of 28 consecutive days.

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### Blood Glucose and Glycosylated Haemoglobin determination

Blood glucose level was measured on days 1, 3, 7, 14, 21 and 28 of treatment. Glucose testing kit was utilized for the measuring of plasma glucose levels in accordance with manufacturers recommended protocols. Blood samples were obtained via tail puncture of the rats. While HbA1c was measured on Day 0 and 28 (Dholi *et al*., *2011; Gandhi & Sasikumar, 2012).*

**Table 1: Blood Glucose Level Changes in AGF Treated Female Wistar Rats with Alloxan-Induced Diabetes**

| Groups /Treatment(s) | Changes in Blood Glucose level (*mmol/L*) | | | | | |
|---|---|---|---|---|---|---|
| | **Day 1** | **Day 3** | **Day 7** | **Day 14** | **Day 21** | **Day 28** |
| **Group 1: Negative control (untreated rats)** | 4.34 ± 0.13 | 4.16 ± 0.25 | 3.74 ± 0.50 | 4.00 ± 0.42 | 4.30 ± 0.15 | 4.30 ± 0.17 |
| **Group 2: Positive control (Alloxan only treated rats)** | 18.26 ± 3.30 [a] | 25.64 ± 2.14 [a] | 22.04 ± 3.81 [a] | 23.28 ± 2.61 [a] | 24.02 ± 1.89 [a] | 27.76 ± 1.39 [a] |
| **Group 3: Alloxan + 250mg/kg AGF** | 23.24 ± 5.03 [a] | 18.24 ± 5.31 [a] | 20.46 ± 5.95 [a] | 17.26 ± 5.66 [a] | 17.58 ± 5.18 [a] | 13.38 ± 5.76 [b] |
| **Group 4: Alloxan + 500mg/kg AGF** | 27.94 ± 1.66 [a] | 20.42 ± 3.48 [a] | 13.44 ± 2.09 | 12.20 ± 2.24 [b] | 8.80 ± 1.31 [b] | 5.94 ± 0.76 [b] |
| **Group 5: Alloxan + 1000mg/kg AGF** | 26.74 ± 1.16 [a] | 21.60 ± 3.27 [a] | 19.64 ± 2.85 [a] | 16.92 ± 2.03 [a] | 13.30 ± 2.06 [b] | 8.48 ± 2.02 [b] |
| **Group 6: Alloxan + Glibenclamide** | 26.12 ± 5.29 [a] | 24.54 ± 2.99 [a] | 18.82 ± 3.32 [a] | 19.16 ± 5.33 [a] | 18.84 ± 5.46 [a, d] | 13.08 ± 5.11 [b] |

Values represent mean ± SEM, n=5; [a] Significant at p<0.05 when compared to group 1; [b] Significant at p<0.05 when compared to group 2. [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4; [e] Significant at p<0.05 when compared to group 5.

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Table 1 shows the results for Blood Glucose Level Changes in AGF Treated Female Wistar Rats with Alloxan-Induced Diabetes

The results for Day 1 indicated significant ($p<0.05$) increase when all groups ( Alloxan only, Alloxan + 250mg/kg AGF, Alloxan + 500mg/kg AGF, Alloxan + 1000mg/kg AGF and Alloxan + Glibenclamide) were compared to group 1 (negative control) respectively. The degree of increase is as follows; group 2 < group 3 < group 6 < group 5 < group 4.

The values for Day 3 showed significant ($p<0.05$) increase when all groups ( Alloxan only, Alloxan + 250mg/kg AGF, Alloxan + 500mg/kg AGF, Alloxan + 1000mg/kg AGF and Alloxan + Glibenclamide) where compared to group 1 (negative control). The degree of increase is as follows; group 3 < group 4 < group 5 < group 6 < group 2

The values for Day 7 showed significant ($p<0.05$) increase in groups 2,3,5 and 6 ( Alloxan only, Alloxan + 250mg/kg AGF, Alloxan + 1000mg/kg AGF and Alloxan + Glibenclamide) when compared to group 1 (negative control). The degree of increase is as follows; group 6 < group 5 < group 3 < group 2.

The values for Day 14 showed significant ($p<0.05$) increase in groups 2,3,5 and 6 ( Alloxan only, Alloxan + 250mg/kg AGF, Alloxan + 1000mg/kg AGF and Alloxan + Glibenclamide) when compared to group 1 (negative control). The degree of increase is as follows; group 5 < group 3 < group 6 < group 2. The values of group 4 (Alloxan + 500mg/kg AGF),was significantly ($p<0.05$) reduced when compared to group 2 (Alloxan only).

The Glucose levels for Day 21 showed that the values of group 2, 3 and 6 ( Alloxan only, Alloxan + 250mg/kg AGF, and Alloxan + Glibenclamide) were significantly ($p<0.05$) increased when compared to group 1 (alloxan only). The values of group 4 and 5 (Alloxan + 250mg/kg AGF and Alloxan + 500mg/kg AGF) were significantly ($p<0.05$) reduced when compared to group 2 (Alloxan only). The values of group 6 (Alloxan + Glibenclamide) were significantly ($p<0.05$) increased when compared to group 4 (Alloxan + 250mg/kg AGF)

For Day 28, the results showed that the glucose levels of group 2 (Alloxan only) were significantly ($p<0.05$) increased when compared to group 1 (negative control) . The values of group 3-6 ( Alloxan + 250mg/kg

AGF, Alloxan + 500mg/kg AGF, Alloxan + 1000mg/kg AGF and Alloxan + Glibenclamide) were significantly($p<0.05$) reduced when compared to group 2 (Alloxan only). The degree of reduction is as follows ; group 6 > group 3 > group 5 > group 4.

**Table 2: Glycosylated Haemoglobin ($HbA_{1c}$) Level Changes in AGF Treated Female Wistar Rats with Alloxan-Induced Diabetes**

| Groups /Treatment(s) | Glycosylated Haemoglobin ($HbA_{1c}$) ( per cent) |
|---|---|
| **Group 1: Negative control (untreated rats)** | 4.10 ± 0.05 |
| **Group 2: Positive control (Alloxan only treated rats)** | 4.64 ± 0.07 [a] |
| **Group 3: Alloxan + 250mg/kg AGF** | 4.30 ± 0.04 [a, b] |
| **Group 4: Alloxan + 500mg/kg AGF** | 4.26 ± 0.06 [a, b] |
| **Group 5: Alloxan + 1000mg/kg AGF** | 4.10 ± 0.04 [b, c, d] |
| **Group 6: Alloxan + Glibenclamide** | 4.08 ± 0.04 [b, c, d] |

Values represent mean ± SEM, n=5; [a] Significant at p<0.05 when compared to group 1; [b] Significant at p<0.05 when compared to group 2. [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4; [e] Significant at p<0.05 when compared to group 5.

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Table 2 shows the results for Glycosylated Haemoglobin ($HbA_{1c}$) Level Changes in AGF Treated Female Wistar Rats with Alloxan-Induced Diabetes

The values for group 2 (Alloxan only) were significantly (p<0.05) increased when compared to group 1 (Negative control). The levels of group 3 and 4 (Alloxan + 250mg/kg AGF and Alloxan + 500mg/kg AGF) were significantly (P<0.05) increased when compared to group 1 (negative control). The values of group 3-6 (Alloxan + 250mg/kg AGF, Alloxan + 500mg/kg HEAG-FP, Alloxan + 1000mg/kg AGF and Alloxan + Glibenclamide respectively) were significantly (P<0.05) reduced when compared to group 2 (Alloxan only). The degree of reduction is as follows; group 3 > group 4 > group 5 > group 6. Groups 5 and 6 (Alloxan + 1000mg/kg AGF and Alloxan + Glibenclamide) were significantly reduced when compared to group 3 and 4 (Alloxan + 250mg/kg AGF and Alloxan + 500mg/kg AGF) respectively.

## 4. DISCUSSION

For the results presented in changes in blood glucose levels, as at day 1 of treatment, blood glucose level of the alloxan induced rats were significantly increased to 18.26 mmol/l as against 4.34mmol/L of the negative control. This is in line with the studies of Fujieda *et al.* (2018) and Lin *et al.* (2019) which reported that alloxan is associated with Diabetes mellitus because it blocks the glucose sensor (glucokinase) in the liver and pancreatic beta cells.

Blood sugar levels were measured on Day 1, day 3, day 7, day 14, day 21 and day 28. Treatment with the herbal extracts in day one didn't yield any marked results. Day 3 and 7 showed some levels of reduction. While there was marked reductions in blood glucose levels from day 14 to 28 with herbal extract having more potency than the synthetic drug (glibenclamide). This effect on blood glucose levels could be as result of the synergistic effect of the five (5) most abundant phytochemicals isolated from *Azanza garckeana* especially, Octadecanic acid , ethyl ester which was reported in the molecular docking result to have a highest binding affinity for the sulfonylurea receptor on the beta cells of the pancreas than even glibenclamide a standard drug in Sulfonylurea class of antidiabetic drugs. These Sulfonylurea class of antidiabetics or secretagogues promote insulin release by closing potassium ion channels therby initiating an action potential in the beta cells that will cause release of insulin (Sunaga *et al.,* 2001). The presence of Triterpene which are said to be anti-diabetic ; can inhibit enzymes involved in glucose metabolism, prevent the development of insulin resistance and normalize plasma glucose and insul-hormon levels ( Nazaruk and Borzym-Kluczyk, 2015) could also be contributory. It does this by regulating the expression of insulin hormone receptor, GLUT 4, and inhibiting alpha amylase and alpha glucosidase activity (Dahiru, 2023).

Glycosylated hemoglobin levels in the negative control was significantly higher in group 2 when induced with alloxan, but decreased markedly when treated with the herbal extract of 250mgkg, 500mgkg and 1000mg kg and when compsred with the synthetic drug. The herbal extract having therefore, more potency than the synthethic drug.

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## 5. CONCLUSION

The study found that alloxan treatment markedly increased blood glucose levels in rats, but the treatments significantly decreased blood glucose levels with the herbal extract showing more potency than the synthetic drug (glibenclamide). This could be due to the presence Triterpenes in the herbal extract, which are anti-diabetic and can inhibit glucose metabolism enzymes, prevent insulin resistance, and normalize plasma glucose and insulin levels. This could also be as a result of the effect of Octadecanoic acid on the SUR-1 receptor.

Glycosylated hemoglobin levels increased with alloxan and decreased with herbal extract, and synthethic drug, with herbal extract showing more potency than synthetic drug.

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## ETHICAL APPROVAL

Prior to commencement of the work, ethical approval was obtained from the University of Port Harcourt Ethics Committee on the 1st of June 2023 with reference number: UPH/CEREMAD/REC/MM89/231. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, 2019). Appropriate measures were taken to ensure the safety and well-being of animal handlers in line with the Animal Handling Safety and Health Procedures (UWA S & H, 2021).

## REFFERENCES

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