

# Evaluation of glycemic index, hypolipidemic and hypoglycemic activities of “*osu une*” on Alloxan induced diabetic rats.

## Abstract

**Background:** The incidence of diabetes has been on the increase due to increase in sedentary lifestyle together with increase in life expectancy. “*Osu une*” is a native meal in Anambra State, Nigeria used in the management of diabetics. There is no scientific based study/data to ascertain the effect on blood glucose level. **Objective:** The study investigated the glycemic index, hypolipidemic and hypoglycemic activities of “*osu une*” on Alloxan induced rats. **Methods:** The “*osu une*” was prepared, dried and blended. Twenty adult male Albino rats were grouped into four of five rats each based on their body weight. Alloxan at a dose of 42mg/kg was induced intravenously through tails of group 2-4 rats. Blood samples were collected from the ocular vein and analyzed for blood glucose and lipid profile on day 7, 14 and 28 using standard method. Ten healthy subjects aged between 24 -40 participated in this study. They were fed with the standard food (50 g glucose) on day one and the test foods on day two, after an overnight fast. Blood samples were taken at 0, 30, 60, 120, and 180 min after the food had been eaten. **Results:** The result showed that rats that received ‘*osu une*’ extract showed an increase in body weight from 130.70mg/kg to 146.20mg/kg, while rats that received glucophage tablets also showed an increase in body weight from 126.01mg/kg to 158.81mg/kg after inducing diabetes. The rats fed ‘*osu une*’ extract had a decrease in fasting blood glucose level, total cholesterol (TC), triglycerides, Low Density Lipoprotein (LDLc) and increase in High Density Lipoprotein (HDLc). The test diet had a low glycemic index of 9.59. **Conclusion:** The study shows that “*osu une*” can play a key role in the management of Diabetes Mellitus.

**Keywords:** glycemic index, hypolipidemia, hypoglycemia, ‘*osu une*’, diabetes, Alloxan.

## 1.1 Introduction

All age groups are affected by the public health issue of diabetes mellitus, however adults aged 50 and older experience it most frequently. Blood glucose (or blood sugar) levels that are elevated in people with diabetes are chronic metabolic conditions that over time cause substantial harm to the heart, blood vessels, eyes, kidneys, and nerves. (WHO, 2023). When the fasting glucose level is greater than or equivalent to 126 mg/mL, it become a chronic metabolic illness (ADA, 2010). Diabetes may result from problems with cellular glucose uptake or an inability of the pancreas to create insulin (Sanofi, 2013). The most prevalent kind of diabetes, type 2, often affects adults and develops when the body stops producing enough insulin or becomes resistant

to it (WHO, 2023). By 2025, there is a universally accepted goal to stop the rise in both diabetes and obesity (WHO, 2023).

WHO (2023) noted that the bulk of the approximately 422 million individuals with diabetes globally reside in low- and middle-income nations, and diabetes is directly responsible for 1.5 million fatalities annually. Over the past few decades, there has been a consistent rise in both the incidence and prevalence of diabetes. According to IFD (2013), there will be 3.7 million diabetes-related fatalities worldwide by 2040, up from 1.5 million in 2012. According to WHO (2016), the number of patients on the African continent is expected to rise from an estimated 32.8 million in 2014 to 41.4 million in 2035. An estimated 2 million deaths were attributed to diabetes and diabetes-related kidney disease in 2019 (WHO, 2023).

The glycemic index (GI) is a number that indicates how much a certain item will raise blood sugar levels. Foods are often rated on a scale of 0-100 and categorized as low (55 or less), medium (56-69), or high (70 and above). The less likely a food is to alter blood sugar levels, the lower its GI is (Racheal, 2020). However, managing diabetic individuals would be greatly aided by low GI diets.

The cost-prohibitive nature of insulin injectable therapy makes it unaffordable for many families in poor nations. Functional foods are preferred over pharmaceuticals in the management of diseases since they are more long-lasting and have fewer or no negative effects. People typically accept their traditional foods with health advantages readily because they are accustomed to them, are familiar with how to prepare them, and enjoy the meals that include them. When properly processed, plant foods with antidiabetic qualities are readily available, more affordable, and have few unfavorable side effects (Guler et al., 2015). The World Health Organization (WHO) also claims that research into the hypoglycemic qualities of medicinal plants has grown in importance (Miaffo et al., 2019). To control diabetes effectively and provide diversity to a patient's diet, it is essential to identify various foods with low glycemic index.

In Anambra State, Nigeria, "Osu une" is a traditional cuisine that is unique to the locals. According to the folktale, the diet is made for the management of diabetes mellitus. "Osu une" is a pudding cooked with crayfish, oil, unripe bananas, salt, spices, and water. The goal of the study is to determine scientifically how the diet affects diabetes control. The extract of "osune" has not been the subject of any prior research on diabetes prevention. This study's goal was to assess

"osu une's" glycemic index, hypoglycemic, and hypolipidemic effects in alloxan-induced diabetic rats.

## **1.1 Materials and method**

### **1.2.1 Test food and processing**

The plant material was 1 kilogram of unripe banana fruit that was bought at the Enugu main market and verified in the herbarium of the Department of Biotechnology and Botany at the University of Nigeria Nsukka. The unripe bananas were cleaned with tap water, the bark was peeled, the bananas were cut into smaller pieces, and an attrition mill was used to smash the pieces into a soft paste. The unripe banana was ground and combined with 10ml red oil, 20g crayfish, 4g seasoning, 5g onions, and 30ml water. Salt was added to taste. The blended paste was tied in small amounts in a folded piece of foil and cooked quickly and evenly for 30 minutes in a pressure cooker until it was hard and knife-free. A few of the samples were baked to dry them out.

### **1.2.2 Chemicals and drugs**

Alloxan and other chemicals used for the study were purchased from a local chemical store at Enugu. Metformin was purchased from a local pharmacy store at Enugu.

### **1.2.3. Animal housing**

Twenty mature male Albino rats weighing between 150 and 153g from the same colony were bought from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, Nigeria. The rats were kept in separate feces and urine-separated metabolic cages. For the rats, a day consisted of precisely 12 hours of light and 12 hours of darkness. The National Research Council's rules for the handling and use of laboratory animals were strictly followed during the conduct of the experiment (National Research Council, 2010). The University of Nigeria Nsukka's Animal Experimentation Ethics Committee granted approval for the use of laboratory animals.

### **1.2.4 Induction of diabetes**

Four groups of five rats each were formed out of the rats. They were fed regular rat food for seven days to help them get used to it. Prior to grouping, the rats underwent a 7-day acclimatization phase and were weighed. After a 12-hour fast, groups 2-4 of animals received

intravenous Alloxan at a dose of 42 mg/kg of body weight in the tail veins to induce diabetes. Throughout the trial, group one received only rat food without an Alloxan. Rat food and 500g of "osu une" were given to group two, rat food and Metformin were given to group three, and rat food alone was given to group four. The standard method was used to examine the body weight, water and food intake, urine production, blood sugar, hemoglobin, total cholesterol, triglycerides, and high and low density lipoproteins. The evolve glucometer was used to measure blood sugar levels. On days 7, 14, and 28 of the trial, the animals' lipid profiles were examined.

### **1.2.5 Blood collection**

Blood was drawn from ocular vein and used for laboratory testing. Using a Dialab kit, a colorimetric enzymatic procedure developed by Trinder (Trinder, 1969) was used to perform a total cholesterol assay. Wiebe *et al.* (1997) described the HDL-c assay utilizing an Inmesco kit. Using a Dialab kit, the triglycerides level was determined using the enzymatic colorimetric method Cole *et al.* (1997) developed. Using the formula provided by Richmond (Richmond, 1973), the LDL-c level was calculated from the other lipids previously acquired.

### **1.2.6 Proximate analysis**

The proximate analysis for moisture, crude protein, crude fibre, fat and ash was carried out using standard methods (AOAC, 2015).

Fifty grammes (50g) of available carbohydrate for the test food sample was calculated from the results of the proximate analysis and the measured portion of the food was served to the subjects. The control diet were administered (50g glucose) in 200ml of distilled water.

### **1.2.7 Determination of blood glucose**

Volunteers for the investigation fasted overnight. They were asked not to perform any strenuous activities or take long walks. They were requested to remain seated for the duration of the test.

Capillary pricked-finger blood samples were taken at baseline (0 min), 30, 60, 90, 120 and 180 mins after consumption of the food. The blood sample was placed immediately on a test strip which was inserted into a calibrated Glucometer (Evolve<sup>R</sup>) which gave direct readings after few seconds.

## **Day 1**

The study started in the morning after an overnight fast by the individuals. A fasting blood sample was taken at 0 min; then after this, the subjects consumed 50 g standard food (50 g of glucose powder dissolved in water) in a comfortable place. The standard food was constituted with 200 ml of water. Blood samples were taken at 30, 60, 120, and 180 min. The blood glucose concentrations were determined immediately using the glucometer.

## Day 2

After an overnight fast, the test foods were consumed by the same group of subjects. Blood samples were taken at 0, 30, 60, 90, and 120 min. The blood glucose concentrations were determined immediately using the glucometer.

The incremental areas under the glycemic response curve were calculated geometrically (Wolever & Jenkins, 1986). The GI was calculated by expressing the glycemic response area for the test food as a percentage of the mean response area of the glucose drink taken by the same subjects. The following formula was applied:

$$GI = \frac{\text{Area under the curve for 50g carbohydrate from test food} \times 100}{\text{Area under the curve for 50g carbohydrate from glucose}}$$

Area under the curve for 50g carbohydrate from glucose

The GI for the food and control was calculated as a mean from the respective average GI of the individuals.

### 1.2.8 Statistical analysis

All results were expressed as mean  $\pm$  SD (Standard Deviation). Statistical analyses were evaluated by one-way ANOVA mean separated using New Multiple Range Test. Statistical significance was accepted at  $p < 0.05$ .

## 1.2 Result

**Table 1** Effect of osu-une 500g on the relative body weight in alloxan-induced diabetic rats g.

Day	Rat chow without diabetic	Rat chow with Mertformin	Rat chow with osu-une	Rat chow alone
7	150.72 <sup>a</sup> $\pm$ 1.16	151.24 <sup>a</sup> $\pm$ 0.48	153.10 <sup>a</sup> $\pm$ 0.35	152.98 <sup>a</sup> $\pm$ 0.81
14	155.24 <sup>a</sup> $\pm$ 0.53	116.01 <sup>b</sup> $\pm$ 1.23	110.70 <sup>b</sup> $\pm$ 0.56	124.60 <sup>b</sup> $\pm$ 2.09
28	152.10 <sup>b</sup> $\pm$ 0.78	158.81 <sup>a</sup> $\pm$ 0.31	146.20 <sup>c</sup> $\pm$ 1.10	110.20 <sup>d</sup> $\pm$ 0.96

Each value is expressed as mean  $\pm$  SD (n = 5).

**Table 2 Effect of osu-une 500 g on fasting blood glucose in alloxan-induced diabetic rats mg/dl .**

Day	Rat chow without diabetic	Rat chow with Mertformin	Rat chow with osu-une	Rat chow alone
7	118.72 <sup>a</sup> $\pm$ 1.54	119.24 <sup>a</sup> $\pm$ 0.38	119.10 <sup>a</sup> $\pm$ 0.31	118.98 <sup>a</sup> $\pm$ 0.18
14	119.24 <sup>b</sup> $\pm$ 0.84	233.01 <sup>a</sup> $\pm$ 1.26	233.70 <sup>a</sup> $\pm$ 0.62	234.60 <sup>a</sup> $\pm$ 0.82
28	118.10 <sup>a</sup> $\pm$ 0.43	121.81 <sup>b</sup> $\pm$ 0.17	126.20 <sup>c</sup> $\pm$ 1.66	240.20 <sup>d</sup> $\pm$ 0.06

Each value is expressed as mean  $\pm$  SD (n = 5).

**Table 3 Effect of osu-une 500 g on Total cholesterol in alloxan-induced diabetic rats (mg/dL).**

Day	Rat chow without diabetic	Rat chow with Mertformin	Rat chow with osu-une	Rat chow alone
7	31.84 <sup>a</sup> $\pm$ 0.18	30.20 <sup>a</sup> $\pm$ 0.15	34.00 <sup>a</sup> $\pm$ 0.04	32.50 <sup>a</sup> $\pm$ 0.72
14	33.00 <sup>b</sup> $\pm$ 0.65	45.10 <sup>a</sup> $\pm$ 0.62	44.50 <sup>a</sup> $\pm$ 0.36	43.90 <sup>a</sup> $\pm$ 0.54
28	30.05 <sup>a</sup> $\pm$ 0.23	34.60 <sup>b</sup> $\pm$ 0.85	37.20 <sup>c</sup> $\pm$ 0.26	44.00 <sup>d</sup> $\pm$ 0.01

Each value is expressed as mean  $\pm$  SD (n = 5).

**Table 4 Effect of osu-une 500 g on Triglyceride in alloxan-induced diabetic rats (mg/dL).**

Day	Rat chow without diabetic	Rat chow with Mertformin	Rat chow with osu-une	Rat chow alone
7	22.10 <sup>a</sup> $\pm$ 0.23	21.00 <sup>a</sup> $\pm$ 0.16	23.00 <sup>a</sup> $\pm$ 0.32	20.90 <sup>a</sup> $\pm$ 0.17
14	23.00 <sup>b</sup> $\pm$ 0.47	35.30 <sup>a</sup> $\pm$ 0.52	34.60 <sup>a</sup> $\pm$ 0.51	33.80 <sup>a</sup> $\pm$ 1.33
28	23.05 <sup>b</sup> $\pm$ 0.08	20.70 <sup>c</sup> $\pm$ 0.63	22.60 <sup>c</sup> $\pm$ 0.91	35.00 <sup>a</sup> $\pm$ 1.66

Each value is expressed as mean  $\pm$  SD (n = 5).

**Table 5 Effect of osu-une 500 g on HDL in alloxan-induced diabetic rats (mg/dL).**

Day	Rat chow without diabetic	Rat chow with Mertformin	Rat chow with osu-une	Rat chow alone
7	12.76a $\pm$ 1.12	12.95a $\pm$ 0.45	13.12a $\pm$ 0.57	13.50a $\pm$ 0.07

14	13.00 a ±0.08	10.20a ±0.21	10.70a ±0.68	9.90a ±1.22
28	13.05c ±0.01	18.30a ±0.32	13.60b ±1.31	6.07b ±0.41

Each value is expressed as mean ± SD (n = 5).

**Table 6: Effect of osu-une 500 g on LDL in alloxan-induced diabetic rats (mg/dL).**

Day	Rat chow without diabetic	Rat chow with Mertformin	Rat chow with osu-une	Rat chow alone
7	16.40 <sup>a</sup> ±0.13	17.35 <sup>a</sup> ±0.67	15.90 <sup>a</sup> ±0.03	16.20 <sup>a</sup> ±0.50
14	16.10 <sup>b</sup> ±0.09	26.70 <sup>a</sup> ±0.45	28.30 <sup>a</sup> ±0.42	26.90 <sup>a</sup> ±0.18
28	16.00 <sup>a</sup> ±0.14	14.10 <sup>a</sup> ±0.11	15.80 <sup>a</sup> ±0.07	27.45 <sup>b</sup> ±0.68

Each value is expressed as mean ± SD (n = 5).

**Table 7: Proximate composition of “osu une”**

Sample	Protein	Ash	Fat	Moisture	Fibre	CHO
Osu une	37.13±0.23	1.27±0.16	2.86±0.32	35.50±43	5.38±0.14	17.86 ±0.39

Mean±SD

**Table 8: The calculated carbohydrate in 100g of prepared food and serving size used for the determination of GI**

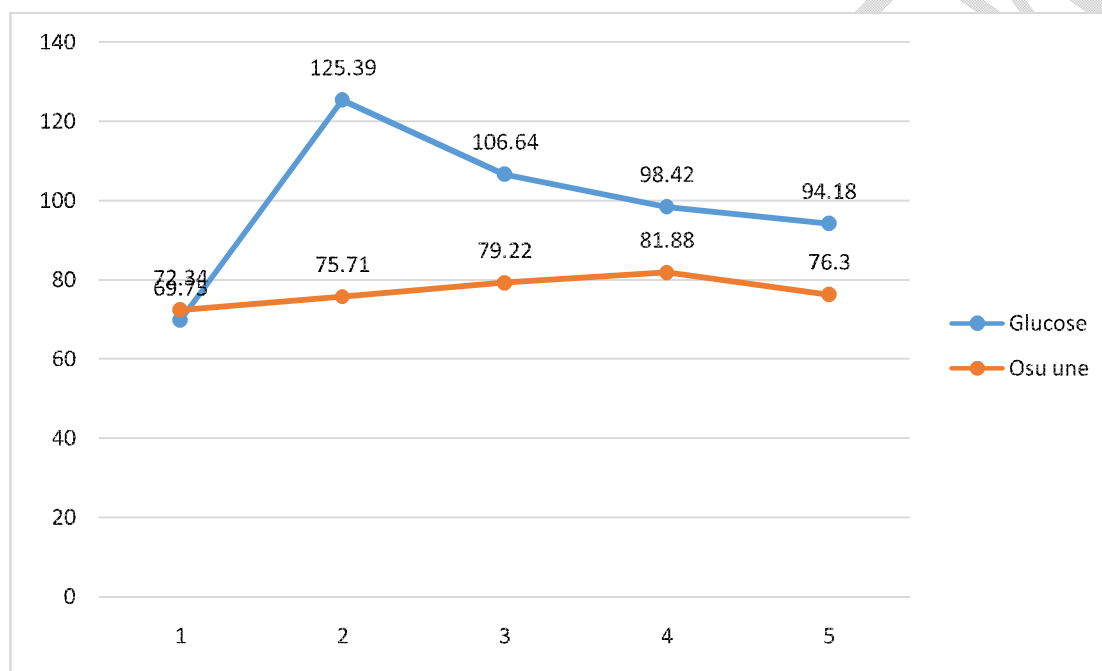
Sample	Calculated CHO in 100g Of prepared food	portion size (g)
Osu une	17.86	280

**Table 9: Blood glucose concentration (mg/dl) of subjects**

Sample	0 mins	30 mins	60 mins	120 mins	180 mins
Glucose	69.75	125.29	106.64	98.42	94.18
Osu une	72.34	75.71	79.22	81.88	76.30

**Table 10: Glycemic index of “*osu une*”**

Sample	Glycemic index	Classification
<i>Osu une</i>	9.59	low



**Fig. 1. Blood glucose concentration of the subjects**

### 1.3 Results and Discussion

There is a tremendous increase in the incidence of diabetes mellitus worldwide. It is necessary to discover other foods with a low glycemic index that will help in the dietary management of diabetes mellitus. The aim of this study is to evaluate the glycemic index and determine the effect of “*osu une* “ on the hypoglycemic and hypolipidemic activities of diabetic-induced rats.

**Body weight:** Table 1 shows the rats' mean body weight. The values ranged from 150.72 to 152.10g on day 7 following acclimatization. On day 7, there is no significant difference in the rats' mean body weight. This is due to the fact that every single rat was healthy and drawn from the same colony. The average weight of the rats ranged from 110.70 to 155.24g on day 14 following the introduction of diabetes. P 0.05 determined that groups 2-4 were significantly different from group 1 due to the absence of alloxan. There is a significant decrease in the body weight of the group 2-4 which were administered with alloxan. These tissues are unable to metabolize blood glucose, hence the loss of body weight seen in the diabetic rats is the result of the hydrolysis of protein (protein turnover) and lipid stores in muscle tissue to provide energy (Pari & Maheswari, 1999). Severe weight loss, perhaps caused by muscular atrophy, is another trait unique to type 1 diabetes (Farida & Shoukry, 1988). Alloxan depletes the muscle and liver, simulating an animal that has been denied a high-protein diet. Sequel of this a diabetic patient is advised to consume a high protein diet. On day 28, the mean body weight ranged between 110.20-158.81g. There is a significant increase in the body weight of the rats of groups 2 and 3 who received Mertformin drug and *osu une* extract. The result of this study is in line with the findings of (Miaffo *et al.*, 2019) that observed a decrease in the relative body weight of rats administered with alloxan and an increase in the relative body weight of rats administered with Mertformin drug to diabetic induced rats. The significant increase in the weight observed in the group that received test diet indicates its effect on the control of muscle atrophy. Whitton & Hems (1975) observed a significant increase in the relative body weight of the rats after administering the extract, which was attributed to its effect on the control of muscle atrophy.

**Fasting blood glucose:** Table 2 shows the mean fasting blood glucose of rats. On day 7, which was the first day after acclimatization the mean fasting glucose level was between 118.72-119.24mg/dl. On day 14, which was the day diabetes was confirmed, the fasting blood glucose was between 119.24-234.60mg/dl. On day 28, which was the last day of treatment/ experiment, the mean fasting blood glucose level was 118.10- 240.20mg/dl. There is a significant increase ( $p < 0.05$ ) in the fasting blood glucose level of rats in groups 2-4 after administering alloxan to the rats to induce diabetes compared to group 1 the normal control. On day 28, there is a decrease in the fasting blood glucose level of rats in groups 2 and 3 that received Mertformin and *osu une* extract. The results of this investigation are consistent with those of earlier studies (Madhuri & Mohanvelu, 2017; Miaffo *et al.*, 2019), which found that rats' fasting blood glucose

levels decreased after they were given plant extract and anti-diabetic medications. The test diet's capacity to regenerate islets of Langerhans cells, transport blood glucose in peripheral tissue, stimulate glucose uptake by peripheral tissues, inhibit endogenous glucose production, and activate gluconeogenesis in the liver and muscles could all be contributing factors to the decrease (Burcelain et al., 1995).

**Lipid profile:** Table 3, 4, 5, and 6 shows the mean total cholesterol, triglyceride, HDL-c, and LDL-c level of rats. On day 7, which was the first day after acclimatization the mean total cholesterol, triglyceride, HDL-c, and LDL-c level were between 30.20- 34.00mg/dl, 20.90- 23.00 mg/dl, 12.76-13.50 mg/dl, and 15.90-17.35 mg/dl. On day 14, which was the day diabetes was confirmed, total cholesterol, triglyceride, HDL-c, and LDL-c level were between 33.00-45.10mg/dl, and 23.00- 25.30mg/dl, 9.90-13.00mg/dl, and 16.10-26.90mg/dl. On day 28, which was the last day of treatment/ experiment, the mean total cholesterol, triglyceride, HDL-c and LDL-c level were 30.05-44.00mg/dl, 20.70- 35.00mg/dl, 6.07- 18.30mg/dl and 14.10-27.45mg/dl. There is a significant increase ( $p < 0.05$ ) in the total cholesterol, triglyceride, and LDL-c level but a significant decrease in the HDL-c level of rats in groups 2-4 after administering alloxan to the rats to induce diabetes compared to group 1 the normal control. Banda *et al.* (2018) observed an increase in TC, TG, VLDL, LDL, and a decrease in HDL in alloxan-induced diabetic rats. In diabetic-induced rats, hyperlipidemia develops as a result of excessive fat mobilization from adipose tissue as a result of inadequate glucose consumption (Akpan et al., 2012). According to studies, the hormone lipase makes it easier to break down stored triacylglycerol into fatty acids, which encourages the liver to turn extra fatty acids into phospholipid and cholesterol. (Rajaei *et al.*, 2015). The diabetic condition renders an enzyme lipoprotein lipase inactive thereby leading to hypertriglyceridemia and a reduction in HDL-c levels (Pushparaj *et al.*, 2007). Krentz (2003) observed that hypertriglyceridemia, hypercholesterolemia and elevated LDL levels are the common factors that lead to the development of atherosclerosis and coronary heart disease in diabetes mellitus patients. On day 28, there is a significant decrease in the TC, TG, LDL-c level as compared to group 4 with an increase in the HDLc level of rats ( $p < 0.05$ ) in groups 2 and 3 that received Mertformin and *osune*. Banda *et al.* (2018) observed that the *L. edulis* administered to diabetic groups had significant reductions in TC, TG, LDL, and VLDL as compared to the diabetic control whilst

HDL levels were significantly increased. This could be attributed to increased utilization of glucose which led to the inhibition of lipid peroxidation and control of lipolytic hormones (Banda *et al.* 2018). Rajaei *et al.* (2015) observed that dietary management and drug therapy that will lead to the lowering of serum lipid and elevation of HDL-c is associated with a decrease in the risk of cardiovascular disease and related complications.

**Glycemic index:** Studies show that different nutritional and physiological factors might have an effect on the blood glycemic response and the GI value of the foods (Omega and Omega, 2018). This may include but is not limited to the digestibility of the starch, interactions of starch with fiber, fat, and protein present, the proportion of the constituent nutrient, and the method of cooking. The low glycemic index of 9.59 of the meal could be attributed to ethnobotanical benefit of ‘*osu une*’ in the community where the meal is predominant. This could also be attributed to the antioxidant and phytochemical content of the test diet.

#### 1.4 Conclusion

In conclusion, “*osu une*” diet with low GI of 9.59 which caused a decrease in fasting blood glucose level, total cholesterol, triglycerides, LDL, and an increase in HDL is recommended for the dietary management of diabetes in a community where the diet is common.

#### Ethical Approval:

The protocol and procedures employed were reviewed and approved by the Ethics Committee of the Enugu State University Teaching Hospital, Parklane Enugu. The procedures followed were also in accordance with the ethical standards of the responsible committee on human experimentation of the Helsinki Declaration of 1975, as revised in 2008.

#### Consent:

Ten (10) healthy human subjects, aged between 24-40 (5 males and 5 females) were selected from the students and staff of the Enugu State University of Science and Technology, Nigeria. They were clinically normal, non diabetic and non-smokers. The subjects were appraised verbally and they gave their informed consent.

**Abbreviations:** ANOVA: Analysis of variance; HDL-c: High-density lipoprotein cholesterol; LDLc: Low density lipoprotein cholesterol; GI: glycemic index

## References

1. American Diabetes Association (2010). "Diagnosis and classification of diabetes mellitus," *Diabetes Care*, vol. 33, no. 1, pp. S62–S69, 2010. View at: [Publisher Site](#) | [Google Scholar](#)
2. Akpan, E. J., Okokon, J. E., & Offong, E. (2012). Antidiabetic and hypolipidemic activities of ethanolic leaf extract and fractions of *Melanthera scandens*. *Asian Pac. J. Trop. Biomed.* 2, 523–527. doi: 10.1016/S2221-1691(12)60089-6
3. AOAC . (2015). Official methods of analysis (15th ed). Washington D.C.: Association of Official Analytical Chemists. [Google Scholar]
4. Banda, M., Nyirenda, J., Muzandu, K., Sijumbila, G. & Mudenda, S. (2018) Antihyperglycemic and Antihyperlipidemic Effects of Aqueous Extracts of *Lannea edulis* in Alloxan-Induced Diabetic Rats *Front. Pharmacol.*, 27 September 2018 | <https://doi.org/10.3389/fphar.2018.01099>
5. Bhandari, M.R. & Kawabata, J. (2004). Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea Spp*) tubers of Nepal. *Food Chem*; 88:163-168
6. Burcelain, R, Eddouks M, Maury J, Kande J, Assan R, & Girard J. (1995) Excessive glucose production rather than insulin resistance accounts for hypoglycaemia in recent onset diabetic rats. *Diabetologia*. 1995;38:283–90
7. Cole, T.G., Klotzsch, S.G., & Mcnamara, J.(1997) Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Dominissak MH, editors. Handbook of Lipoprotein testing. Washington: AACC Press; 1997. p. 26–115.
8. Farida A, & Shoukry M (1988). The lipid lowering effect of an anti-diabetic plant extract. *Acta Diabetol Lat.* 1988;25(1):1–5. 29. Whitton PD, Hems DA. Glycogen synthesis in perfused liver of streptozotocin diabetic rats. *Biochem J.* 1975;21(2):150–3.
9. Guler, E., Manav, E., & Ugurlu, E. (2015) Medicinal plants used by traditional healers in Bozuyuk (Bilecik–Turkey). *J Ethnopharmacol.* 2015;17:339–47
10. IFD.(2013) Diabetes Atlas, 6th edition,

11. Krentz, A. J. (2003). Lipoprotein abnormalities and their consequences for patients with Type 2 diabetes. *Diabetes Obes. Metab.* 5, 19–27. doi: 10.1046/j.1462-8902.2003.0310.x CrossRef Full Text | Google Scholar
12. Madhuri, A.S. & Mohanvelu, R.(2017) Evaluation of Antidiabetic Activity of Aqueous Extract of *Mangifera Indica* Leaves in Alloxan Induced Diabetic Rats. *Biomed. & Pharmacol. J.*, Vol. 10(2), 1029-1035 (2017)
13. Miaffo, D., Kamgue, O.G. , Tebou1, N.L , Temhoul, C.M. & Kamanyi, A.(2019) Antidiabetic and antioxidant potentials of *Vitellaria paradoxa* barks in alloxan-induced diabetic rats. *Clinical Phytoscience* 5:44 <https://doi.org/10.1186/s40816-019-0141-z>
14. National Research Council,(2010) *Guide for the Care and Use of Laboratory Animals*, National Academies Press, Washington, DC, USA, 2010
15. Nwnkwo, M.O, Etim, E.E. & Ogbonna, I.O. (2018). Investigation on the Anti-diabetic Activity of *Sphenostylis stenocarpa* Seed Milk Extract in Alloxan-induced Diabetes Rats, *International Journal of Scientific and Research Publications*, Volume 8, Issue 8, August 2018 824 ISSN 2250-3153
16. Omage, K.,& Omage, S.O. (2018). Evaluation of the glycemic indices of three commonly eaten mixed meals in Okada, Edo State. *Food Sci Nutr.* 2018;6:220–228. <https://doi.org/10.1002/fsn3.550> [PMC free article] [PubMed] [Google Scholar]
17. Pari, L.& Maheswari, J (1999). Hypoglycaemic effect of *Musa sapientum* L. in alloxaninduced diabetic rats. *J Ethnopharmacol.* 1999;68(1–3):321–5.
18. . Pushparaj, P. N., Low, H. K., Manikandan, J., Tan, B. K., & Tan, C. H. (2007). Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 111, 430–434. doi: 10.1021/np0202428
19. Racheal, M.S. (2020). Glycemic Index: What it is and how to use it. [www.healthline.com](http://www.healthline.com)
20. Rajaei, Z., Hadjzadeh, M.-A.-R., Moradi, R., Ghorbani, A., & Saghebi, A. (2015). Antihyperglycemic and antihyperlipidemic effects of hydroalcoholic extract of *Securigera securidaca* seeds in streptozotocin-induced diabetic rats. *Adv. Biomed. Res.* 4:33. doi: 10.4103/2277-9175.150427 [PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)
21. Richmond, W.(1973) Preparation and properties of cholesterol oxidase from *Nucurdia* sp. and its application to enzymatic assay of total cholesterol in serum. *Clin Chem.* 1973;19:1350–6
22. Sanofi.(2013) *Diabetes: a global epidemic*, 2013.

23. Trinder, P (1969). Quantitative in vitro determination of cholesterol in serum and plasma. *Ann Clin Biochem.* 1969;6:24–7.
24. Wiebe, D.A., & Warnick, G.R. (1997) Measurement of high-density lipoprotein cholesterol. In: Rafai N, Warnick GR, Sunday MH, editors. *Hand book of lipoprotein testing.* Washington: AACC Press; 1997. p. 9–107.
25. Whitton, P.D. & Hems, D.A.(1975) Glycogen synthesis in perfused liver of streptozotocin diabetic rats. *Biochem J.* 1975;21(2):150–3. [Google Scholar](#)
26. WHO (2016). Alert on the rise in the number of diabetics in the world, 2016