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Hepatoprotective Effect of Ethanolic Leaves Extract of *Carica papaya* (Pawpaw) on Alloxan-Induced Diabetic Wistar Rats

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ABSTRACT
The hepatoprotective effects of Carica papaya's ethanolic leaves extract were evaluated. Twenty-five Wistar rats were divided into five groups. Diabetes was induced via intraperitoneal injection of alloxan (150 mg/kg body weight) after 16 hours of fasting. Blood glucose levels were checked 72 hours post-induction. Rats with levels ≥200 mg/dL were considered diabetic. The groups consisted of: (I) normoglycemic control, (II) diabetic non-treated, (III) diabetic treated with metformin (10 mg/kg), and (IV-V) diabetic treated with 200 and 400 mg/kg of the extract, respectively. Treatments lasted 21 days. Blood samples were collected via cardiac puncture, and serum was analyzed for liver enzymes, total protein, bilirubin, and albumin. Results showed significant decreases ($p < 0.05$) in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, direct bilirubin, total bilirubin, and blood glucose levels in groups treated with 200 and 400 mg/kg of the extract. Significant increases ($p < 0.05$) in total protein and albumin were also observed. This study demonstrates the ethanolic leaves extract of Carica papaya's antidiabetic and hepatoprotective effects in alloxan-induced diabetic Wistar rats.

Keywords:
Diabetes mellitus,
hepatoprotection,
liver enzymes,
carica papaya.

INTRODUCTION

Diabetes mellitus is a long-term metabolic disorder characterized by persistent hyperglycemia (high blood sugar) resulting from the body's inability to produce or effectively use insulin, a hormone that regulates blood sugar levels. There are two main types of diabetes: type 1, which is caused by an autoimmune reaction that destroys insulin-producing cells, and type 2, which is the most prevalent and is often associated with lifestyle factors such as obesity and physical inactivity (American Diabetes Association, 2009). Diabetes if left unchecked can result in complications to the eyes, kidneys, nerves and cardiovascular system, significantly reducing quality of life and increasing the risk of premature mortality. High blood sugar levels (hyperglycemia), a hallmark of uncontrolled diabetes, affect blood vessels throughout the body. This microvascular damage which is also referred to as diabetic microangiopathy, leads to a group of complications. Diabetic retinopathy develops when blood vessels in the retina deteriorate, that results in poor blood circulation, potentially causing blindness; Diabetic nephropathy involves progressive kidney damage that gradually deteriorate kidney function and can culminate in kidney failure; lastly, diabetic neuropathy affects the nerves, resulting in symptoms such as numbness, pain, and weakness (Dahl, 2007). Furthermore, diabetes significantly increases the risk of cardiovascular diseases, including heart attacks, strokes and peripheral arterial disease, due to the development of atherosclerosis and other vascular complications (American Heart Association, 2023). Globally, the prevalence of diabetes has been steadily rising, with the International Diabetes Federation (IDF) estimating that 463 million adults were living with the condition in 2019. This figure is projected to increase to 700 million by 2045 if current trends continue. Africa is also not exempted from this epidemic, with the IDF reporting a diabetes prevalence of 3.9% among adults aged 20-79 years in the African region in 2019 (IDF, 2019). Nigeria, the most populous country in Africa, has also recorded a significant rise in diabetes prevalence in recent decades. A systematic review and meta-analysis by Uloko *et al*., 2018 found an overall pooled prevalence of 5.77% among Nigerian adults, with the highest rates observed in the south-south geopolitical zone (9.8%). The authors also identified several risk factors for diabetes in Nigeria, including a family history, urban dwelling, unhealthy dietary habits, cigarette smoking, older age, physical inactivity and obesity (Uloko *et al.*, 2018). A more recent study

estimated that Nigeria had 2.2 million adults with diabetes in 2019, a figure projected to reach 4.1 million by 2045 (IDF, 2019). The rising prevalence of diabetes in Nigeria and across Africa is a major public health concern, as it threatens to overwhelm already strained healthcare systems and exacerbate socioeconomic inequalities. Effective strategies for the prevention, early detection and management of diabetes are urgently needed to mitigate the impact of this growing epidemic.

The liver plays a critical role in glucose metabolism and regulation, making it an essential for diabetes management. A healthy liver helps maintain blood glucose levels within the normal range and protects against excessive fluctuations, which is vital since both hyperglycemia (high blood sugar) and hypoglycemia (low blood sugar) can be detrimental to health (Adeva-Andany *et al*., 2016). The liver stores glucose as glycogen and releases it into the bloodstream when needed, such as between meals or during exercise, to keep blood sugar stable (Centers for Disease Control and Prevention (CDC), 2022). However, diabetes can negatively impact liver health, with up to 70% of people with type 2 diabetes also having non-alcoholic fatty liver disease (NAFLD). NAFLD occurs when fat accumulates in the liver, affecting its function. If left unchecked, it can progress to more severe forms like non-alcoholic steatohepatitis (NASH), which involves inflammation and liver damage (CDC, 2022). Poorly controlled diabetes increases the risk of NAFLD and other liver diseases, such as cirrhosis and liver cancer. Conversely, liver disease can complicate diabetes management. Altered metabolism, drug interactions, and liver toxicity can make it challenging to control blood sugar levels in those with diabetes-related liver disease (Max Healthcare, 2022). Liver damage may impair the liver's ability to store and release glucose, leading to greater blood sugar fluctuations.

Hepatoprotective agents are essential in mitigating liver damage associated with diabetes by reducing oxidative stress, inhibiting inflammatory pathways and promoting liver cell regeneration. Natural compounds, including flavonoids, polyphenols and saponins, have demonstrated hepatoprotective properties in various studies. For example, research indicates that morin, a flavonoid, effectively modulates oxidative stress and improves carbohydrate metabolism in diabetic models, showcasing its potential as a hepatoprotective agent (Kochuvelickakathu *et al.*, 2022).

Carica Papaya, commonly known as pawpaw or papaya, is a tropical fruit-bearing plant native to Central America and widely cultivated in tropical and subtropical regions around the world. This plant is not only valued for its sweet, nutritious fruit but also for its extensive medicinal properties, which have been utilized in traditional medicine for centuries (Koul *et al*., 2022). The leaves, seeds, and fruit of *Carica papaya* have been used in various cultures to treat different diseases, making it a significant component of herbal medicine.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from Bingham University Ethical Committee for the Use and Care of Laboratory Animals.

Materials

Animal feed, water, ethanol, Alloxan monohydrate,universal containers, set of dissecting kit, glucometer and test strips, sample bottles, weighing balance, needle and syringes, cotton wool, animal cages, drinkers, sawdust, bench centrifuge, plane sample bottles, pipettes and biochemical analyzer.

Experimental Animals

Twenty five (25) male Wistar rats weighing between 160g to 200g were procured from the Animal House, College of Medicine, University of Ibadan, Oyo State. The rats were housed in well aerated plastic cages with wood shavings (sawdust) as bedding in the Animal Care Unit of the Faculty of Basic Medical Sciences, Bingham University, Karu, Nasarawa State. They were fed with standard commercial pellet diet and water ad libitum; and were acclimatized for two weeks before the commencement of the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

Plant Collection and Identification

Fresh *Carica Papaya* leaves were harvested Auta-Balefi Karu Local Government Area of Nasarawa State. The leaves were properly washed with water to remove sand and other impurities, and were taken to the Department of Biological Sciences, Faculty of Science and Technology Bingham University, Karu, for identification and authentication.

Plant Preparation and Extraction

The fresh *Carica papaya* leaves were air dried andgrounded using a pestle and mortar, the powder was obtained. The groundedpowder was macerated in ethanol (70%) at a ratio of 5ml/g of the powder with continuous stirring for three days at 4^{o}C as described by Tanko *et al* (2014) modifiedby Mallo *et al.* (2023). The extract was filtered and lyophilized to obtain a semi-solid powder about (200 g), which was stored at 4^{o}C.

Experimental Induction and Determination of Diabetes Mellitus

Diabetes mellitus was induced after fasting the rats for 16 hours. The animals were given a single *intra-peritoneal* injection of alloxan monohydrate 150 mg/kg body weight as described by Nwakanma *et al*. (2022) mordified by Mallo et al (2023). The alloxan

monohydrate was dissolved in ice-cold 0.1 M sodium citrate buffer and was followed by oral administration of 2-3 ml sucrose solution 10% (w/v) (Lanjhiyana *et al*., 2011). Animals were fasted overnight and one drop blood sample was obtained by pricking the lateral tail vein using sterile surgical scissors and immediately the blood glucose levels were determined. Fasting blood glucose level was determined by using the glucose oxidase method as described by Trinder (1969), modified by Kumar*et al* (2018). Animals with blood glucose levels above 200 mg/dl were considered to be diabetic (Kim *et al*., 2008).

Experimental Design

All the rats were fed with animal feed and water for 21 days, the rats were randomly divided into five groups of five (n = 5) rats each as follows:

Group 1: This served as an experimental control (non-diabetic) group.

Group 2: This served as the diabetic untreated group.

Group 3: The rats in this group were diabeticand treated with 10 mg/kg body weight metformin (Palaksha *et al*., 2020).

Group 4: The rats in this group were diabeticand treated with 200 mg/kg body weight ethanolic leaves extract*of Carica papaya* orally.

Group 5: The rats in this group were diabeticand treated with 400 mg/kgbody weight ethanolic leaves extract*of Carica papaya* orally

Blood glucose level was determined at the end of 21 days treatment period

Sample collection and preparation

The animals were then sacrificed by anaesthetizing under chloroform inhalation and dissected. Blood samples were collected from the heart using the cardiac puncture method and transferred into plain blood sample bottles. The samples were then centrifuged at 3000rpm for 10 minutes to obtain the serum. The serum was used to assay for levels of AST, ALT, ALP, TP, DB, TB and ALB.

i. Alkaline phosphatase (ALP)
ii. Alanine aminotransferase (ALT)
iii. Aspartate aminotransferase (AST)
iv. Total protein
v. Direct bilirubin
vi. Total bilirubin

Data Analysis

The data obtained from the study was analyzed using one way Analysis of Variance (ANOVA). A statistical package SPSS version 25 was used and the results obtained were presented as Mean ± Standard error of mean followed by a post *hoc test* of Turkey to determine the level of statistical significance and p values ($p < 0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

Table 1: Blood Glucose Level

Groups	Initial Blood Glucose (mg/dl)	Final Blood Glucose (mg/dl)
Control	116.83 ± 4.50a	87.60 ± 2.94[a]
Diabetic control	341.75 ± 93.28[b]	285.20 ± 12.11[b]
Diabetic + 10mg/kg Met.	343.40 ± 67.16[b]	152.85 ± 4.70[a]
Diabetic + 200mg/kg of CP	346.75 ± 40.80[b]	161.20 ± 9.42[a]
Diabetic + 400mg/kg of CP	486.75 ± 94.02[b]	149.00 ± 5.91[a]

The superscripts ([a], [b]) shows statistical significance ($p < 0.05$).

Table 2: Liver Enzymes

Groups	ASTIU/L	ALT IU/L	ALP IU/L
Control	30.72 ± 1.67[a]	60.23 ± 6.50[a]	70.00 ± 1.62[a]
Diabetic control	58.80 ± 2.34[b]	66.50 ± 1.39[b]	145.77 ± 3.80[b]
Diabetic + 10mg/kgMet.	37.63 ± 1.68[a]	39.20 ± 0.84[a]	73.86 ± 2.20[a]
Diabetic + 200mg/kg of CP	37.93 ± 0.84[a]	39.82 ± 0.79[a]	100.81 ± 3.59[a]
Diabetic + 400mg/kg of CP	31.29 ± 1.16[a]	34.71 ± 1.28[a]	75.37 ± 2.18[a]

The superscripts ([a], [b]) shows statistical significance ($p < 0.05$). ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase

Table 3: Bilirubin, Albumin and Total Protein

Groups	TP umol/L	DB umol/L	TBumol/LALB umol/L
Control	70.00±1.62[a]2.97±0.37[a]	15.50±1.70[a]	41.36±2.92[a]
Diabetic control	58.52±1.54[b]6.74±0.56[b]	28.46±2.84[b]	28.29±2.23[b]
Diabetic + 10mg/kg Met.	75.58±0.85[a]2.32±0.08[a]	18.14±1.10[a]	43.31±0.73
Diabetic + 200mg/kgCP	74.14±1.29[a]4.27±0.11[a]	18.84±0.66[a]	46.20±1.29[a]
Diabetic + 400mg/kg CP	68.69±1.88[a]2.13±0.12[a]	10.72±1.04[a]	39.75±0.61[a]

The superscripts ([a], [b]) shows statistical significance ($p < 0.05$). TP = total protein, DB = direct bilirubin, TB = total bilirubin, ALB = albumin, Met. = metformin, CP = *Carica papaya*.

From Table 1, the result of blood glucose levels it was observed that there was a statistical significant ($p < 0.05$) decreased in blood glucose level in the groups treated with both 200 mg/kg and 400 mg/kg body weight of *Carica papaya* leaves extract (161.20 ± 9.42 and 149.00 ± 5.91) compared to the diabetic untreated group.The reduction in fasting blood sugar (FBS) levels among the treated rats suggest that ethanol extract of *Carica papaya* enhance insulin sensitivity and also increased beta cells proliferation and regeneration, which promote glucose uptake in peripheral tissues. This is consistent with finding by Sinha *et al*. (2018), who reported that *Carica papaya* leaves extract can reduces blood glucose levels in diabetic rats. The decrease in blood glucose levels caused by the extract was in a dose-dependent manner, with the (400mg/kg body weight) showing the more potent effect than the 200 mg/kg body weight.

In Table 2, the results demonstrate the significant hepatoprotective effects of *Carica papaya* lead crude ethanol extract in alloxan-induced diabetic male wistar rats. The observed improvements in liver enzymes levels, including AST, ALT and ALP, suggest that the extract possesses potent protective properties against diabetes-induced liver damage. The hepatoprotective effects observed in the study aligns with the anti-inflammatory and antioxidant properties reported by Owoyele *et al*., 2008; Pandey *et al.,* 2016; and Aulianshah *et al.*, 2024. By reducing inflammation, *Carica papaya* extract may help reserve liver function and prevent the release of these enzymes into the bloodstream.

The results from this study revealed that diabetes mellitus increases the activities of liver enzymes, this was evident by a statistically significant ($p < 0.05$) increased in serum levels of liver enzymes (AST, ALT and ALP) compared to the control groups. The results for the liver function from this study indicated that both the 200 mg/kg and 400 mg/kg body weight of the ethanol extract of *Carica papaya* leaves produced a statistical significant ($p < 0.05$) decreased in in AST (37.93 ± 0.84 and 31.29 ± 1.16) compared to the diabetic non-treated rats (58.80 ± 2.34), there was also statistical significant ($p < 0.05$) decreased in serum ALT levels on treatment of the diabetic rats with 200 and 400 mg/kg body weight of the the ethanolic extract of *Carica papaya* leaves extract (39.82 ± 0.79 and 34.71 ± 1.28) compared to the diabetic rats which remains elevated (66.50 ± 1.39). There was also a statistically significant (p , 0.05) decreased in serum ALP on treatment with both 200 and 400 mg/kg body weight of the ethanolic extract of Carica papaya leaves extract (100.81 ± 3.59 and 75.37 ± 2.18) compared to the diabetic control group (145.77 ± 3.80).

Liver function tests give information about the state of the liver, describing its functionality (albumin), cellular integrity (transaminases) and its link with biliary tract (ALP) (Ezejiofor *et al*., 2013). Thapa and Anuj (2007) had reported that standard range of accepted values for liver function tests, beyond which liver damage may be suspected is ALT (10 – 55 µ/L), AST (10 – 40 µ/L), and ALP (45 – 115 µ/L). Kamal and Hessah (2015) corroborated this when they reported that rise in AST, ALT and ALP values beyond this limits indicate early diagnosis of hepatotoxicity and tissue damage. It has been reported that liver toxicity is associated with increase in various serum liver enzymes resulting from damage to the hepatocytes. The statisticallysignificance decreased in the serum levels of AST, ALT and ALP revealed that the hepatoprotective effect of the ethanolic leaves extract of *Carica papaya*.

In Table 3, the results for total protein and albumin showed a statistical significant increased ($p < 0.05$) in the rats treated with both 200 and 400 mg/kg body weight of the extract of *Carica papaya* leaves as compared to the diabetic non-treated rats which showed a significant ($p < 0.05$) decreased in total protein and albumin. However, there was a significant increase in both direct bilirubin and total bilirubin in the diabetic untreated rats compared to the control rats and treatment with both 200 and 400 mg/kg body weight of the ethanolic leaves extract of *Carica papaya* produced a significant ($p < 0.05$) decreased in both the direct and total bilirubin concentration.

The hepatoprotective effects of *Carica papaya* leaf extract can be attributed to its rich phytochemical composition, which includes flavonoids, alkaloids and phenolic compounds known for their antioxidant properties. These compounds can mitigate oxidative stress and inflammation, both of which are critical contributors to liver damage in diabetic models

(Sharma *et al*., 2022). The antioxidant activity of *Carica papaya* leaf extract helps to neutralize free radicals, thereby protecting hepatocytes from oxidative damage and supporting normal liver function. Pandey *et al*., 2016 noted that the use of herbal remedies in diabetes management could provide additional benefits beyond glycemic control, including hepatoprotection.

CONCLUSION

The findings of the study revealed that diabetes mellitus induces liver damage which was associated with elevation in liver enzymes and bilirubin concentration, however, the ethanolic leaves extract of *Carica papaya* exhibits an antidiabetic and hepatoprotective properties which was evidenced by lowering of blood glucose levels ameliorating changes in liver damage biomarkers in alloxan-induced diabetic Wistar rats.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCE

Adeva-Andany, M. M., Pérez-Felpete, N., Fernández-Fernández, C., Donapetry-García, C., & Pazos-García, C. (2016). Liver glucose metabolism in humans. *Bioscience Reports*, 36(6), e00416. DOI:10.1042/BSR20160385

American Diabetes Association.(2009). Definition and Classification of Diabetes Mellitus. *Diabetes Care*, 44(1), S62-S67

American Heart Association.(2023). Heart and Stroke Statistics—2023 Update: A Report from the American Heart Association. *Circulation*, 147(8). DOI: 10.1161/CIR.0000000000001123

Aulianshah, V., Thaharah, Y. R., Zakiah, N., & Handayani, R. (2024). Anti-Inflammatory Effect of *Carica Papaya* Leaves Extract In Male Wistar Rats Based On Variation Of Concentration. *International Journal of Health and Pharmaceutical*, 4(3), 503-507.

Centers for Disease Control and Prevention.(2022). Types 2 diabetes and your liver. International Diabetes Federation. (2019). *IDF Diabetes Atlas*, 9

Dahl-Jorgensen K.(2007). Diabetic microangiopathy. *Acta Paediatrica*, 87(425) 31-34. DOI: https://doi.org/10.1111/j.1651-2227.1998.tb01249.x

Ezejiofor C N, Orish C N and Orish E B. (2013). Effect of aqueous leaves extract of Costus afer on the liver and kidney of male albino wistar rats. *Anc Science Life*,33(1): 4 – 9. doi: 10.4103/0257-7941.134554. PMID: 25161323; PMCID: PMC4140021.

International Diabetes Federation. (2019). Diabetes Atlas, 9th Edition. Brussels, Belgium: International Diabetes Federation.

Kamal, A A and Hessah M A. (2015). Alterations – lipid profile, oxidative stress and hepatic function in rats fed with saccharine and methyl salicylates. *International Journal of Clinical Experimental Medicine*, 8(4): 6133 – 6144.

Kim H, Jeong D, Jung H, Yokozawa T, Choi J (2008). Hypolipidemic effects of Sophora flavescens and its constituents in Poloxamer-407 inducedhyperlipidemic and cholesterol-fed rats. *Biol. Pharm. Bull*, 31 (1): 73 – 78. doi: https://doi.org/10.1248/bpb.31.73

Kochuvelickakathu, S.S., Neelakanta P.P.S., Sukanta, M. & Saraswathy, M. (2022). Hepatoprotective effect of morin via regulating the oxidative stress and carbohydrate metabolism in STZ induced diabetic rats. *Bioactive Compounds in Health and Disease*. DOI: https://doi.org/10.31989/bchd.v5i3.893

Koul, B., Pudhuvai, B., Sharma, C., Kumar, A., Sharma, V., Yadav, D., & Jin, J.O. (2022). Carica papaya L.: A tropical fruit with benefits beyond the tropics. Diversity, 14(8), 683: DOI: https://doi.org/10.3390/d14080683

Kumar, V., Gill, K. D., Kumar, V. and Gill, K. D. (2018). Estimation of blood glucose levels by Glucose Oxidase Method. DOI: https://doi.org/10.1007/978-981-10-8186-6_13

Lanjhiyana, S., Garabadu, D., Ahirwar, D., Bigoniya, P., Rana, A. C., Patra, K. C. and Karuppaih, M. (2011). Hypoglycemic activity studies on root extracts of Murraya koenigii root in Alloxan-induced diabetic rats. *J Nat Prod Plant Resour*, *1*(2), 91-104.

Mallo, M. J., Danborno, A. M., Musa, S. A., Jimoh, A., Toryila, J. E., Soretire, T. G. and Tanko, Y. (2023). Effects of Ethanolic Extract of Cyperus esculentus (Tiger Nut) Tubers on Blood Glucose Level and Lipid Profile in Alloxan-Induced Diabetes Mellitus in Male Wistar Rats. *Journal of Basics and Applied Sciences Research*, *1*(1), 64-70. DOI: https://doi.org./10.33003/jobasr-2023-v1i1-21

Max Healthcare (2022). Diabetes and liver. All you need to know. Max Hospital NAFLD with diabetes. *Hepatology*, 64(5), 1384-1394

Nwakanma, A.A., Ekon, M.B., Ngwuben, I.C., Idaguko, C.A. and Elemuo, C.O. (2022). Cyperus

esculentus L. Protects Testis and Sperm Morphology of Hyperglycaemic Rats. *International Journal of Medical and Surgical Sciences,* 9(3), 1-16. DOI: 10.32457/ijmss.v9i3.1924

Owoyele, B., Olubori, M., Funmilayo, A.A., & Ojuawo A.O. (2008). Anti-inflammatory activities of ethanolic extract of *Carica Papaya* leaves. *Inflammopharmacology*, 16(4), 168-173. DOI: 10.1007/s10787-008-7008-0

Palaksha, M. N., Mani, T. T., Manjunatha, E., & Kumar, G. P. (2020). Comparative study of In-Vivo effects of Glipizide and Metformin HCl on plasma concentration of Aminophylline in healthy rabbits. *Asian Journal of Pharmaceutical Research*, *10*(2), 62-66.

Pandey, S., Cabot, P. J., Shaw, P. N., & Hewavitharana, A. K. (2016). Anti-inflammatory and immunomodulatory properties of *Carica Papaya*. *Journal of Immunotoxicology*,13(4), 590-602. DOI: https://doi.org/10.3109/1547691X.2016.1149528

Sharma, A., Sharma, R., Sharma, M., Kumar, M., Barbhai, M. D., Lorenzo, J. M., & Naushad, M. (2022). Carica papaya L. Leaves: Deciphering Its Antioxidant Bioactives, Biological Activities, Innovative Products, and Safety Aspects. *Antioxidants*, 11(6), 1040. DOI: https://doi.org/10.1155/2022/2451733

Sinha, A., & Shahbaz, M. (2018). Estimation of environmental Kuznets curve for CO2 emission: role of renewable energy generation in India. *Renewable energy*, *119*, 703-711.

Tanko, Y, Mohammed A, Mabrouk, M.A ,Fatihu M.Y & Musa K.Y. (2014). Effects of N-Butanol And Ethylacetate Fractions of Indigofera Pulchra on Serum Lipid Peroxidation and Anti-Oxidant Enzymes on Normoglycaemic and Alloxan-Induced Diabetic Wistar Rats. *Annals of Biological Sciences* 2 (2):58-65

Thapa, B. R., & Walia, A. (2007). Liver function tests and their interpretation. *The Indian Journal of Pediatrics*, *74*, 663-671.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of clinical Biochemistry*, 6(1), 24-27.DOI: https://doi.org/10.1177/000456326900600108

Uloko, A. E., Musa, B. M., Ramalan, M. A., Gezawa, I. D., Puepet, F. H., Uloko, A. T., Borodo, M. M., & Sada, K. B. (2018). Prevalence and risk factors for diabetes mellitus in Nigeria: A systematic review and meta-analysis. *Diabetes Therapy*, 9(3), 1307-1316. DOI: https://doi.org/10.1007/s13300-018-0441-1