Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

Effects of Ethyl Acetate Leaf Extract of *Annona muricata* on some Enzymes of Carbohydrate Metabolism in Streptozotocin-Induced Diabetic Wistar Rats

Abubakar Z. Lawal [1*], Halima A. Abdulrahim[1], Yusuf A. Iyanda[2], Ibrahim Munirudeen[3], Adekunle, A. Sanni[4]

[1] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Kwara, Nigeria*
[2] *Department of Environmental Management and Toxicology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria*
[3] *Department of Medical Laboratory Sciences, Kwara State University, Malete, Nigeria,*
[4] *Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Oyo, Nigeria*

OPEN ACCESS

***CORRESPONDENCE**
Lawal, A.Z.
lawal.za@unilorin.edu.ng

ARTICLE HISTORY
Received: 28/12/2021
Reviewed: 11/01/2022
Revised: 23/02/2022
Accepted: 08/03/2022
Published: 31/03/2022

CITATION
Lawal, A.Z., Abdulrahim H.A., Iyanda, Y.A., Munirudeen I. and Sanni, A.A. (2022). Effects of ethyl acetate leaf extract of *Annona muricata* on some enzymes of carbohydrate metabolism in streptozotocin-induced diabetic wistar rats. *Nigerian Journal of Biochemistry and Molecular Biology*. 37(1), 87-92

ABSTRACT

Diabetes is a chronic metabolic disease of multiple etiologies characterized by high blood sugar levels. The management of diabetes is taken as a global problem and curative treatment is yet to be uncovered. The leaf, root and bark of *Annona muricata* have been reportedly used locally as an antidiabetic agent. This study aimed to evaluate the effects of ethylacetate leaf extract of *Annona muricata* (AMLE) on selected enzymes of carbohydrate metabolism in diabetic rat model**.** Twenty male rats weighing 180 to 220 g were randomly assigned into four groups. Groups A and B were non-diabetic and diabetic rats respectively, treated with 5% dimethyl sulfoxide saline respectively. Groups C and D were diabetic rats treated with 200 mg/kg body weight (b.wt) AMLE and 5 mg/kg b.wt glibenclamide respectively for 14 days. Diabetes was induced by a single dose of 45 mg/kg b.wt streptozotocin (STZ) intraperitoneally. Rats with blood glucose values above 13.9 mmol/l 48 hours after STZ injection were considered diabetic. Animals were sacrificed on day 15 and hepatic activities of glucose-6-phosphate dehydrogenase, glucokinase, glucose-6-phosphatase and lactate dehydrogenase were assayed. The levels of hepatic lactate dehydrogenase, glucose-6-phosphate dehydrogenase and glucokinase activities in AMLE treated diabetic rats were significantly ($p<0.05$) reduced compared to untreated diabetic rats. However, the activity of glucose-6-phosphatase was only slightly inhibited in the diabetic rats treated with AMLE. Ethylacetate leaf extract of *A. muricata* inhibited the activities of glucose-6-phosphatase and lactate dehydrogenase in this study. These findings partially support the use of this plant in the treatment of diabetes.

Keywords: *Hepatic, glucose-6-phosphatase, streptozotocin, DMSO, Annona muricata*

INTRODUCTION

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves (WHO, 2020). Diabetes may present with symptoms such as thirst, polyuria, weight loss, and blurring of vision. In most severe cases, diabetic ketoacidosis (DKA) or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective management, death (WHO, 1999). The high blood glucose concentration and other biochemical irregularities results from a ß-cells deficiency of the endocrine pancreas and/or from a reduced sensitivity to insulin in target cells (Wua *et al.*, 2011). Disturbance in carbohydrate metabolism and consistent efforts of the physiological systems to correct the imbalance constitute an excess exertion on the endocrine system resulting in the deterioration of endocrine control.

The progressive deterioration of endocrine system

exacerbates metabolic disturbances by altering carbohydrate metabolic enzymes activities resulting primarily to a high blood sugar level (Fava, 2008). The major enzymes that play critical roles in liver glucose homeostasis include glucokinase and glycogen synthase which catalyze the process of glycogenesis; and the key enzymes responsible for the regulation of gluconeogenesis are glucose-6-phosphatase, pyruvate carboxylase, phosphoenolpyruvate carboxylkinase, and fructose-1, 6-bisphosphatase (Eid *et al.*, 2006). Glucokinase, a hexokinase isozyme, is a monomeric allosteric enzyme and is mainly expressed in hepatocytes and pancreatic β-cells (Cornish-Bowden and Cardenas, 2004). Glucokinase plays a central role in blood glucose homeostasis through its unique kinetic character by enhancing glycogen synthesis, in hepatocytes (Agius, 2008), converting glucose to glucose-6-phosphate, and sensing glucose for insulin secretion in pancreatic βeta cells (Matschinsky, *et al.*, 1993). Mutations and structural defects in glucokinase are directly associated with type II diabetes mellitus and maturity-onset diabetes of the young type II diabetes (Matschinsky, 2009). Thus, glucokinase has been an important molecular target for studying blood glucose homeostasis and developing antidiabetic drugs (Matschinsky, 2011). Glucose-6-phosphatase found mainly in the kidneys and the liver, plays an important role in producing glucose during fasting and starvation. Glucose-6-phosphatase is involved in the final step of gluconeogenesis, the production of glucose from non-carbohydrate carbon substances and glycogenolysis, where it completes the conversion of glucose-6-phosphate to glucose, for body utilization. This enzyme is a target of insulin action, where it is inhibited in states of hyperglycemia to prevent the production of glucose. Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme of the pentose phosphate pathway. It plays a central role in cell metabolism and was found to play pathophysiologic roles in many diseases like diabetes, aldosterone-induced endothelial dysfunction, and cancer. Glucose-6-phosphate dehydrogenase catalyzes the first NADPH- generating reaction in Pentose Phosphate Pathway (PPP). Lactate dehydrogenase (LDH) is the enzyme involved in the final step of anaerobic glycolysis. Various tetrameric LDH isoenzymes are expressed in different tissues, and are composed of LDH-A and LDH-B subunits. Of these, only the A-type is expressed in purified pancreatic β-cells (Sekine *et al.*, 1994). The role of LDH in the regulation of glucose metabolism is determined by various factors, such as the absolute activity level of LDH, the glycolytic flux initiated by glucokinase/hexokinase and the complex process of channeling of glycolytic products to aerobic mitochondrial metabolism (Oscar *et al.*, 2000).

Medicinal herbs with antidiabetic activities are increasingly being sought by diabetic patients and health-care professionals as an alternative approach. Most of the existing synthetic diabetes therapies have limited efficacy, limited tolerability, and/or significant mechanism-based side effects (Rotenstein *et al.*, 2012). It is still difficult to attain adequate glycemic control amongst many diabetic patients due to the progressive decline in β-cell function, despite the existing pharmacotherapy (Wallace and Matthews, 2000). Herbs and other alternative therapies commonly used, that are less likely to have the side effects of modern drugs for diabetes mellitus, have been explored (Dey *et al.*, 2002). The leaf, root and bark of *Annona muricata* have been reportedly used locally as an anti-diabetic agent in the Peruvian Amazon, African and Asia. Phytochemical investigations on different parts of the *Annona Muricata* plant have shown the presence of various compounds and phytoconstituents (Adewole and Caxton-Martins, 2006). Therefore, this study was designed to provide valuable scientific information on the effects of ethyl acetate leaf extract of *Annona muricata* on some enzymes of carbohydrate metabolism in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Chemicals/Reagent

All the chemicals and reagents used were of standard analytical grade.

Plant preparation and extraction

Annona muricata leaves were collected from Oja Tuntun, Ilorin, Kwara state, Nigeria. The leaves were identified and authenticated at the Department of Plant Biology, University of Ilorin and a voucher specimen (UILH/003/1106) was deposited in the herbarium. The leaves were air dried and crushed into smaller pieces using mortar and pestle. The plant sample was weighed and extracted with n-hexane, ethylacetate and methanol using a serial exhaustive extraction method according to Das *et al.* (2010). The *in-vitro* antioxidants test was carried out on the extracts and the ethylacetate fraction which had the highest antioxidant activity selected for this study (Lawal *et al.*, 2019).

Experimental animals

Twenty (20) healthy male Wistar rats weighing 200 to 250 g were used in this study. They were housed under standard laboratory conditions of light, temperature and relative humidity. The animals were given standard rat pellets and water *ad libitum*. All the experimental procedures were carried out in accordance with the recommendation of National institute of Health Guide for the care and use of Laboratory animals (NIH, 1985) as well as Ethical

Guidelines for the use of Laboratory Animals in LAUTECH, Nigeria.

Induction of diabetes mellitus

The animals were fasted overnight and diabetes induced experimentally by intraperitoneal administration of a single dose of 45 mg/kg body weight (8.6 mg in 1 ml physiological saline) of a freshly-prepared streptozotocin (STZ) Al-Hariri *et al*., 2011. The rats were allowed access to 5% glucose solution overnight to overcome the STZ-induced hypoglycaemia. The rats in the control group were injected with normal saline. Estimation of fasting blood sugar (FBS) was done 72 hours after STZ induction using Fine Test Glucometer. Blood sample for the FBS was obtained from sterilized rat's tail vein and blood glucose values above 13.9 mmol/l were considered diabetic.

Experimental design

Twenty (20) from already acclimatized rats, were randomly distributed into a group of non-diabetic and three groups of STZ-diabetic rats. Each of the four groups consisted of 5 rats. Groups A and B were non-diabetic and diabetic control rats respectively, treated with vehicle (5% DMSO saline); Group C was diabetic rats treated with AMLE (200mg/kg b.wt) in 5% DMSO saline and Group D was diabetic rats treated with glibenclamide (5 mg/kg b.wt) in 5% DMSO saline. Treatment were administered orally once daily for 14 days.

Animal sacrifice and tissue preparation

Rats were sacrificed after 12 hours fasting on the 15th day under anaesthesia and liver tissue portion for each rat were excised, rinsed in ice-cold saline and homogenized in phosphate buffer (pH 7.4). The homogenates were centrifuged at 3000 rpm and the supernatant used for assay of selected hepatic enzymes of carbohydrate metabolism.

Assay of glucokinase activity

Glucokinase activity was measured as previously described (Zhang *et al.*, 2009; Davidson and Arion, 1987). Activity of glucokinase was assayed using glucokinase reagents ($MgCl_2$ buffer, Glucose, ATP, NAD, G-6-PDH) obtained from Fortress diagnostic, UK, by an enzymatic kinetic UV rate method. The measurement is based upon the reduction of NAD^+ through a coupled reaction with glucose-6-phosphate dehydrogenase and is determined by measuring the increase in absorbance at 340 nm using spectrophotometer. Exactly10µl of the sample was reacted with 200µl of diluted glucokinase solution, mixed thoroughly and incubated at 30°C for 7 minutes. The change in absorbance was monitored at 340 nm. Glucokinase activities were calculated as mU/mg protein and then expressed in U/L. Protein concentration was measured according to the method of Lowry *et al*. (1951).

Assay of glucose-6-phosphatase activity

Glucose-6-phosphase activity was assayed using glucose-6-phosphatase kits (glucose-6-phosphatase buffer, Glucose 6-Phosphate/Substrate, Trichloroacetic Acid, Phosphorus Standard Solution, Sulfuric Acid Solution, Ammonium Molybdate Solution, Taussky-Shorr Color Reagent) obtained from Sigma-Aldrich. With Product Number B9754 (Baginsky *et al.*, 1974) by estimation of inorganic phosphate (Pi) librated from glucose-6-phosphate. Exactly 200µl of the glucose-6-phosphatase enzyme solution was added to 10µl of sample, mixed and incubated at 37 ^{0}C for 5 minutes. The change in absorbance was monitored at 340 nm. Glucose-6-phosphatase activities was calculated and expressed in units per liter (U/L).

Assay of glucose-6-phosphate dehydrogenase activity

Glucose-6-Phosphate dehydrogenase activity was measured using glucose-6-phosphate kits (R_1: co-enzyme-substrate, R_2: Buffer, R_3: lysis reagent) obtained from Spectrum Diagnostics, Cairo, Egypt, with product code: 372001 by the method described by Angelo *et al.* (2009). Glucose-6-Phosphate dehydrogenase catalyzes the oxidation of glucose-6-phosphate with the reduction of $NADP^+$ to NADPH. The rate of reduction of $NADP^+$ to NADPH is measured as an increase in absorbance which is proportional to the glucose-6-phosphate dehydrogenase activity in the sample. Exactly 200µl of the glucose-6-phosphate dehydrogenase solution was added to 10µl of sample, mixed and incubated at 30 ^{0}C. The change in absorbance was monitored at 340 nm. Glucose-6-phosphate dehydrogenase activities was calculated and expressed in units per litter (U/L).

Assay of lactate dehydrogenase activity

Lactate dehydrogenase (LDH) activity was assayed using lactate dehydrogenase reagents (R_1: Buffer/substrate, R_2: NADH) obtained from Biorex diagnostic, UK, with product code: BX0242 by the method previously described by Wrobledwski and La Due (1995). Lactate dehydrogenase catalyses the transformation of pyruvate to lactate; NADH is oxidized to NAD^+ in the reaction process. The rate of decrease in NADH is directly proportional to the LDH activity and is estimated by measurement of the rate of absorbance change at 340 nm due to the reduction. Exactly 10µl of the sample was reacted with 200µl of the reagent and incubated at 37^{o}C for 5 minutes. The change in absorbance was monitored at 340nm. Lactate dehydrogenase activities was calculated and expressed in units per liters (U/L).

Statistical analysis

Data obtained are expressed as mean ± standard error of mean (SEM) of 5 determinations. For statistical analysis, data were subjected to one-way analysis of variance (ANOVA) and followed by post hoc test. GraphPad prism version 8.0 was used for the statistical analysis. The data were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Results

The activity of glucose-6-phosphate dehydrogenase (Figure 1) was observed to be significantly increased in group B rats (diabetic control) compared to the normal control (group A). Treatment of diabetic rats (group C) with 200 mg/kg body weight of AMLE resulted in significant decrease ($p< 0.05$) in the activity of glucose-6-phosphate dehydrogenase when compared with the diabetic control (group B).

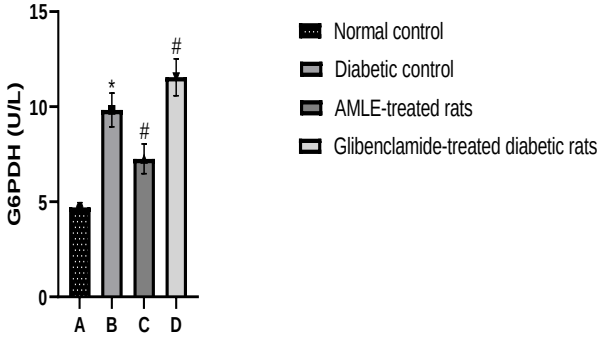

Figure 1. Effects of Ethyl Acetate Leaf Extract of *Annona muricata* on Rat Hepatic Glucose-6-phosphate Dehydrogenase Activity.

Values are mean ± SEM (n=5); *p< 0.05 compared with normal control, #p< 0.05 compared with diabetic control.

Glucose-6-phosphatase activity (Figure 2) of diabetic control (group B) was significantly increased ($p< 0.05$) compared to that of normal control (group A). Treatment of diabetic rats (group C) with 200 mg/kg body weight of AMLE caused significant decrease ($p< 0.05$) in glucose-6-phosphatase activity when compared with that of the diabetic control rats (group B). However, treatment with glibenclamide significantly increases glucose-6-phosphatase activity when compared with AMLE treated rats. The activity of glucokinase (Figure 3) in group B rats (diabetic control) shows no significant difference when compared to the normal control (group A). Treatment of diabetic rats (group C) with 200 mg/kg body weight of AMLE caused significant ($p< 0.05$) decrease in glucokinase activity compared to that of untreated diabetic rats group (group B). However, treatment with 5 mg/kg body weight glibenclamide resulted in significant increase in glucokinase activity when compared with group B rats (diabetic control) and group C (AMLE treated). Lactate dehydrogenase activity (Figure 4) of diabetic control rats (group B) was significantly increased ($p< 0.05$) compared to that of normal control (group A). The activity of lactate dehydrogenase in the treated diabetic rats' groups C and D revealed no significant difference when compared with the untreated diabetic rats (group B).

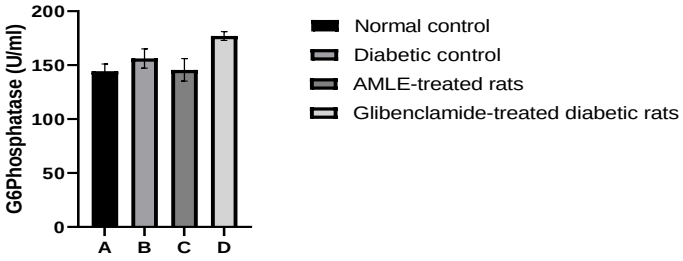

Figure 2. Effects of Ethyl Acetate Leaf Extract of *Annona muricata* on Rat Hepatic Glucose-6-phosphatase Activity.

Values are mean ± SEM (n=5). *p< 0.05 compared with normal control, +p< 0.05 compared with diabetic control.

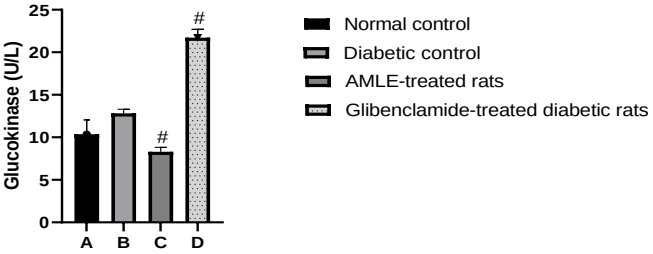

Figure 3: Effects of Ethyl Acetate Leaf Extract of *Annona muricata* on Rat Hepatic Glucokinase Activity.

Values are mean ± SEM (n=5). *p< 0.05 compared with normal control, #p< 0.05 compared with diabetic control.

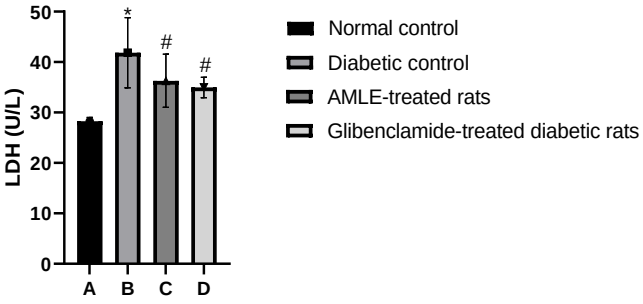

Figure 4. Effects of Ethyl Acetate Leaf Extract of *Annona muricata* on Rat Hepatic Lactate Dehydrogenase Activity.

Values are mean ± SEM (n=5). *p< 0.05 compared with normal control, #p< 0.05 compared with diabetic control

Discussion

Glucose-6-phosphate dehydrogenase is important in the production of reduced glutathione which is a major agent in the control of oxidative stress condition. Oxidative stress is reported to predispose normal individuals to diabetes (Moussa, 2008). Alterations in glucose-6-phosphate dehydrogenase activity can significantly alter oxidative stress-induced cell death (Vulliamy and Mason, 1992). Previous studies have shown that liver Glucose-6-phosphate dehydrogenase activity is significantly reduced in diabetic experimental animals and humans (Gupta *et al*., 1997; Zhang *et al.*, 2000). It has been reported that hyperglycemia decreases G6PD activity in the liver by increasing protein kinase activity, leading to an increase in oxidative stress. In addition, one of the major factors responsible for diabetic complications has been shown to be an increase in oxidative stress due to a decrease in G6PD activity (Zhang *et al.*, 2000). In the present study, treatment of diabetic rat with AMLE significantly reduced the G6PD activity. However, glibenclamide administration significantly increased the glucose-6-phosphate dehydrogenase activity in group D. This implies that the mechanism of antidiabetic effects of *AMLE* was not due to G6PD enzyme activity. In diabetic condition, glucose-6-phosphatase activity is elevated and symptoms associated with hyperglycemia are exacerbated (Kolawole and Akanji, 2014). Glucose-6-phosphatase plays an important role in the regulation of the gluconeogenic pathway. The increase in the activity of Glucose-6-phosphatase in untreated diabetic rats leads to an excessive amount of hepatic glucose production and contributes to the elevated blood glucose levels (Van de Werve *et al.*, 2000). In this study, AMLE administration resulted in decrease in hepatic activity of glucose-6-phosphatase. This is probably a reflection of hypoglycemic effect of the extract. Impairment of glucokinase activity plays a major role in precipitating glucose intolerance and the development of diabetes (Zhang *et al*., 2009). The activity of glucokinase is impaired in the liver and pancreas due to the damage caused by the reactive oxygen species that are generated by streptozotocin in diabetic rats (Srinivasan and Ramarao, 2007; Zhang *et al*., 2009). An increase in the activity of glucokinase in *glibenclamide*-treated diabetic rats implied that the cellular entry of glucose was facilitated by glibenclamide, which in turn stimulated the activity of the enzyme. It was observed that antidiabetic activity of AMLE was not supported by its effect on glucokinase activity. Lactate dehydrogenase (LDH) acts through oxidation process on pyruvate to be converted into lactate, which is subsequently converted to glucose in gluconeogenic flux and it is a functional enzyme in anaerobic glycolysis (Das *at al.,* 2010). Increased LDH activity in diabetes mellitus has been reported by Pozzilli *et al.* (1997) and Prentki *et al.* (1977). Administration of *AMLE* and glibenclamide significantly decreased LDH activity towards the normal, probably as a result of the stimulation of the oxidation of NADH. Normal LDH activity is indicative of improved channeling of (pyruvate) glucose for mitochondrial oxidation.

CONCLUSION

In conclusion, the findings in this present study support the use of this plant in the treatment of diabetes.

AUTHORS' CONTRIBUTIONS

Conceptualization, AZL; Design, methodology and investigation AZL and AAS; Acquisition of data AZL. and IM; Analysis, HAA Interpretation, YAI.; Writing – Original draft preparation, AZL.; Writing-review and editing, AZL, YAI and IM. All authors have read and agreed to the published version of the manuscript.

FUNDING STATEMENT

The authors received no financial support for the research, authorship, and publication of this article

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Adewole, S.O. and Caxton-Martins, E.A. (2006). Morphological changes and hypoglycemic effects of *Annona muricata* Linn. (*Annonaceae*) leaf aqueous extract on pancreatic B-cells of streptozotocin-treated diabetic rats. *African Journal of Biomedical Research*, 9(3), 173-187.

Agius L (2008). Glucokinase and molecular aspects of liver glycogen metabolism. *Biochemical Journal*. 414(1):1–18

Cornish-Bowden, A., and Cardenas, M. L. (2004). Glucokinase: a monomeric enzyme with positive cooperativity. Glucokinase and glycemic disease: From Basics to Novel Therapeutics, 125–134. doi:10.1159/000079011

Das, K., Tiwari, R.K.S. and Shrivastava, D.K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: current methods and future trends. *Journal of Medicinal Plants Research*, 4(2), 104-111.

Dey, L., Attele, A.S. and Yuanm C.S. (2002). Alternative therapies for type 2 diabetes. *Alternative Medicine Review*, 7(1), 45-58.

Eid, A., Bodin, S., Ferrier, B., Delage, H., Boghossian, M., Martin, M., Baverel, G. and Conjard, A. (2006). Intrinsic gluconeogenesis is enhanced in renal proximal tubules of Zucker diabetic fatty rats. *Journal of the American Society of Nephrology* 17(2), 398–405.

Fava, S. (2008). Role of postprandial hyperglycemia in cardiovascular disease. *Expert Review in Cardiovascular Therapy*. 6(6), 859-872.

Gupta, B. L., Nehal, M. and Baquer, N. Z. (1997). Effect of experimental diabetes on the activities of hexokinase, glucose-6-phosphate dehydrogenase and catecholamines

in rat erythrocytes of different ages. *Indian Journal of Experimental Biology*, 35(7), 792–795.

Lawal, Z. A., hamid, A.A., Shehu, A., God'shelp, E., Ajibade, O. S., Zubair, O. A., Ogheneovo, P., Mukadam, A. A. and Adebayo, C. T. (2017). Biochemical properties, *in-vitro* antimicrobial, and free radical scavenging activities of the leaves of *Annona muricata*. *Journal of Applied Sciences and Environmental Management*, 21(6), 1197-1201.

Matschinsky FM (2009). Assessing the potential of glucokinase activators in diabetes therapy. *Nature Reviews Drug Discovery*. 8(5):399–416

Matschinsky, F.M., Liang, Y., Kesavan, P., Wang, L., Froguel, P., Velho, G., et al. (1993). Glucolinase as pancreatic B-cell sensor and diabetes gene. *Journal of Clinical Investigation*. 92, 2092-2098. Doi:10.1172/JC1116809

Oscar A, Markus T and Sigurd (2000). Importance of lactate dehydrogenase for the regulation of glycolytic flux and insulin secretion in insulin-producing cells. *Biochemical Journal, 352(2), 373–*. doi:10.1042/0264-6021:3520373.

Pozzilli, A., Signore, R. D. G., Leslie K., Alberti, P., Zimmet, R. and De Fronzo, H. (1997). "Infections, immunity and diabetes," in *International Text Book of Diabetes Mellitus*. Eds., pp. 1231–1241, 2nd edition, 1997.

Prentki, M., Tornheim, K. and Corkey, B. E. (1997). Signal transduction mechanisms in nutrient-induced insulin secretion. *Diabetologia*, 40 Suppl 2, S32–S41. https://doi.org/10.1007/s001250051395

Rotenstein, L.S., Kozak, B.M., Shivers, J.P., Yarchoan, M., Close, J. and Close, K.L. (2012). The ideal diabetes therapy: *Clinical Diabetes*, 30(2), 44–53.

Sekine, N., Cirulli, V., Regazzi, R., Brown, L. J., Gine, E., Tamarit-Rodriguez, J., Girotti, M., Marie, S., MacDonald, M. J., Wollheim, C. B. and Rutter, G.A. (1994). Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic beta-cells. Potential role in nutrient sensing. *Journal of Biological Chemistry*, 269(7), 4895–4902.

Srinivasan, K. and Ramarao, P. (2007). Animal models in type 2 diabetes research: an overview. *The Indian Journal of Medical Research*, 125(3), 451–472.

Van de Werve, G., Lange, A., Newgard, C., Méchin, M. C., Li, Y. and Berteloot, A. (2000). New lessons in the regulation of glucose metabolism taught by the glucose 6-phosphatase system. *European Journal of Biochemistry*, 267(6), 1533–1549. doi:10.1046/j.1432-1327.2000.01160.x

Vulliamy, T., Mason, P. and Luzzatto, L. (1992). The molecular basis of glucose-6-phosphate dehydrogenase deficiency. *Trends in Genetics* 8(4), 138–143. https://doi.org/10.1016/0168-9525(92)90372-B

Wallace, T.M. and Matthews, D.R. (2000). Poor glycaemic control in type 2 diabetes: a conspiracy of disease, suboptimal therapy and attitude. *Quarterly Journal of Medicine*, 93(6), 369–374.

World Health Organization (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. *Report of a WHO Consultation*. Part 1: diagnosis and classification of diabetes mellitus, Geneva.

World Health Organization-*Diabetes Fact Sheets*, 2020

Wua, C., Li, Y., Chena, Y., Laoa, X., Shenga, L. and Daia, R. (2011). Hypoglycemic effect of *Belamcanda chinensis* leaf extract in normal and STZ-induced diabetic rats and its potential active faction. *Phytomedicine;* 18(4), 292-297.

Zhang, X., Liang, W., Mao, Y., Li, H., Yang, Y. and Tan, H. (2009). Hepatic glucokinase activity is the primary defect in alloxan-induced diabetes of mice. *Biomedicine and Pharmacotherapy*, 63(3), 180–186. https://doi.org/10.1016/j.biopha.2007.07.006

Zhang, Z., Apse, K., Pang, J. and Stanton, R. C. (2000). High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. *The Journal of Biological Chemistry*, 275(51), 40042–40047. https://doi.org/10.1074/jbc.M007505200