IJNRD.ORG ISSN : 2456-4184

INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Diabetes type 2: Evaluating the effects of Claramine on Insulin- Mediated Glucose Regulation in Hepatoma Cell Line.

[1]LAMIDI, Tajudeen Babatunde and [2]KILEDU, Israel Ahmadu

[1]Principal Lecturer, [2]Senior Lecturer

[1]Science Laboratory Technology (SLT)

[1] Federal Polytechnic, PMB 05 Bali, Taraba state, Nigeria

Abstract: This study has been undertaken to investigate the effects of claramine on insulin-mediated glucose regulation in Hepatoma (HepG2) cell lines. Research materials including Hep G2 cell line, T-75 flasks, Eppendorf tubes, 96-well plates, Bradford reagent test kit, cell culture media (Dulbecco's Modified Eagle Medium) (DMEM) and all reagents for buffer preparation was purchased. First experiment was conducted to test the viability of the HepG 2 cell lines using standard procedure. Second round of experiment was conducted to culture the HepG 2 cells at 37°C. The cells were divided into 6 wells plates and allowed to adhere for 24 hours. After adherence, the cells were treated with high glucose and different concentrations of insulin and claramine. The determination of glucose utilisation by the cells was done with multiscan-FC spectrophotometer at 540nm and the amount of glucose utilised by the cell was calculated and use for protein normalisation. Combination of 50nm of insulin with various concentrations of claramine showed trend in the uptake of glucose by Hep2 cells with highest uptake at 2.0um of claramine. However, with 100nm insulin there was relative little and irregular variation in glucose uptake by HepG 2 cells with different concentrations of claramine. Also, glucose utilisation with respect to joint variation of insulin and claramine concentrations showed increase uptake at 50nm insulin and 1um claramine.

Keywords: Diabetes Type 2, Insulin, Hepatoma cell line, Claramine, PTB1B

INTRODUCTION

Diabetes mellitus is the third leading cause of death (after heart disease and cancer) in many developed countries, affecting 6 – 8% of the general population. It is a clinical condition characterised by increased blood glucose level (hyperglycaemia) due to insufficient or inefficient (incompetent) insulin. That is, insulin is either not produced in sufficient quantity or inefficient in its action on the target tissues. As a consequence, the blood glucose level is elevated

beyond the range of 3.5 – 8 m-mol/litre (63 – 144 mg/100ml) which spills over into urine (1,2). Among other risk factors, it leads overtime to serious damage to the heart, blood vessels, eyes, kidneys and nerves (3)

Protein Tyrosine Phosphatise IB (PTPIB), specifically expressed in various human tissues, as member of PTP family is a modulator of insulin signalling pathways. It negatively regulates the leptin and insulin by dephosphorylation and deactivation of insulin receptors thereby switching off insulin signal transduction (4). Studies have shown that the roles of PTPIB is critically related to obesity and diabetes. The important roles of PTP1B related to obesity and diabetes were confirmed by a deletion of PTP1B gene in mice (5). It has been reported that PTPIB inhibitors might play an important role as efficient therapeutic target for type-II diabetes and obesity by increasing insulin and leptin sensitivity. In human, it is located at chromosome 20q13 (6).

Insulin is a polypeptide hormone consisting about 50 amino acids secreted by the β-cells of the pancreatic islets of Langerhans that constantly maintains the normal blood glucose levels by controlling cellular uptake of glucose, regulating carbohydrate, lipid and protein metabolism and facilitating cell division and growth through its effects on mitogens (1). Insulin increases glucose uptake in the muscles and adipose tissues by stimulating the translocation of glucose transporter GLUT4 from the interior to the cell surface and inhibits glucose production in the liver cells and skeletal muscles (glycogenesis) and therefore serve as the primary regulatory molecules of the blood glucose levels. It also promotes lipogenesis in adipose tissues and enhances synthesis of protein (1).

The normal value of sugar in human blood stream is estimated at 90mg/100cm^3 even after the heaviest carbohydrate meal rarely exceeds 150mg/100cm^3. After entering the hepatic portal vein on absorption from the small intestine into the bloodstream, it is conveyed to the liver. Instead of being metabolised or stored, it may pass on from the liver to the general circulation (7).

In recent years, many pharmaceutical companies have developed various PTP1B inhibitors as drug candidates for therapy of Type 2 diabetes in clinical trials, including claramine and trodusquemine (Sun, et. al., 2016). Claramine is a spamino group-containing polyaminosteroid derivative which possess selective inhibitory activity against PTPIB. It has been reported that claramine and trodusquemine causes the activation of key components of insulin signalling pathway in cultured neuronal cell line with high level phosphorylation of insulin receptor-β (IR-β), Akt and GSK3β (8).

Human hepatocellular carcinoma (HepG2) cells are non-tumorigenic cell line which were developed in order to minimize the use of experimental animals for toxicological assays and maintain specie-specificity, the cell line present very high rate of proliferation and has epithelial-like morphology (8).

The prevalence of type-2 diabetes has become epidemic and is one of the world's most common disease (9). There is currently inadequate therapeutic approach against the underlying causes as well as complications associated with the disease. This calls for an urgent development of novel strategies that would tackle the menace and reduce the rates of mortality associated with type-2 diabetes around the globe.

The inhibitory action of PTPIB on insulin cell surface receptors has proven to be the underlying cause of type 2 diabetes (10). Report from (10) shows that inhibition of insulin receptors or deficiency of PTPIB results in glucose

hypersensitivity. Claramine on the other hand has selective inhibitory activity on PTPIB which could be a target therapeutic agent against type II diabetes mellitus

The aim of this research work is to evaluate the effects of claramine on insulin-mediated glucose regulation in HepG2 cell line. The objectives of this research work include: Evaluating the glucose uptake by HepG2 cells after treatment of cells with various concentrations of insulin; Evaluating the glucose uptake by HepG2 cells after treatment of cells with constant concentration of insulin and various concentrations of claramine and evaluating the glucose uptake by HepG2 cells after treatment of cells with various concentrations of insulin and various concentrations of claramine;

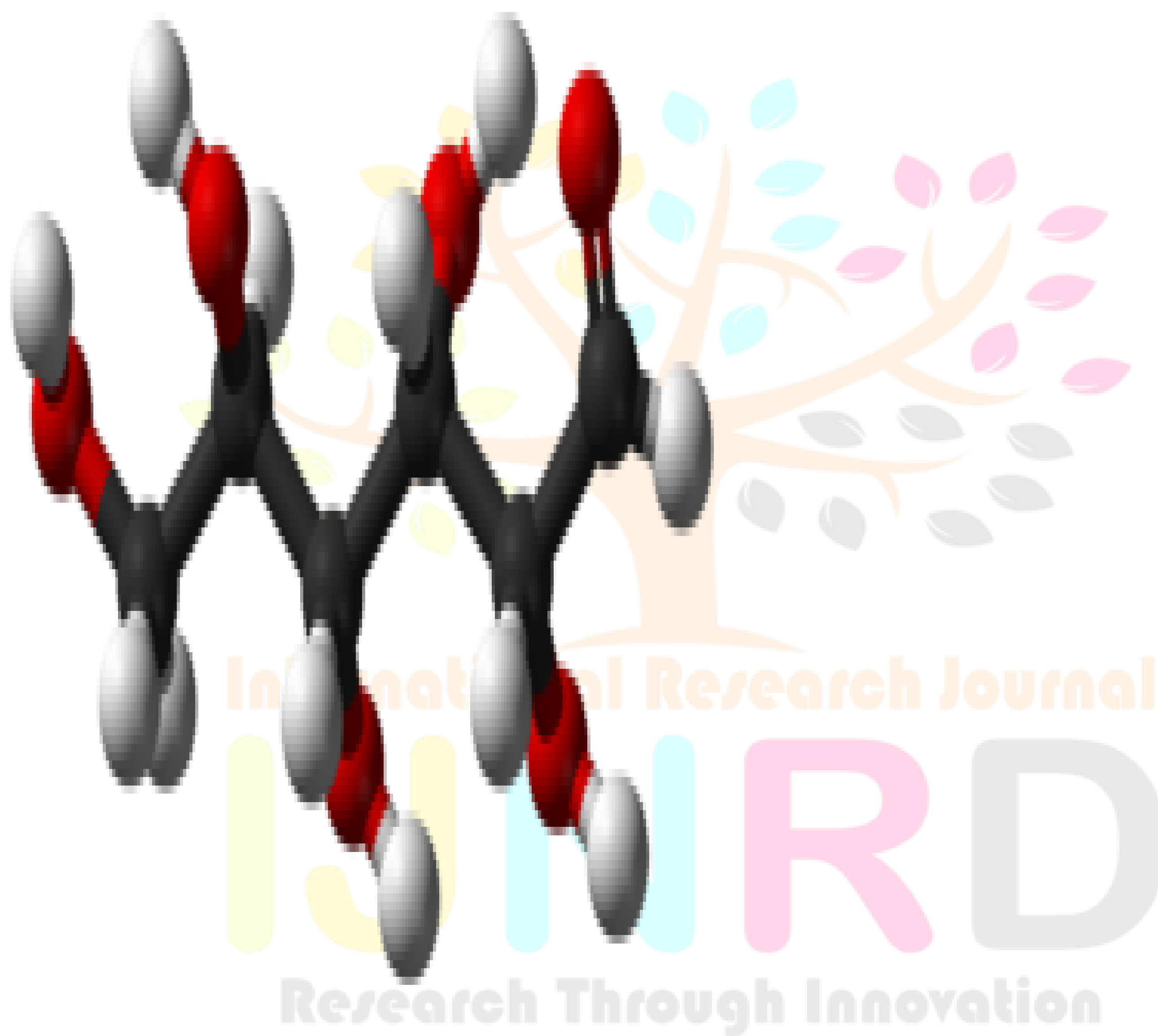

Fig. 1: Ball-and-stick model of a glucose molecule (www.wikipedia.org/wiki/diabetes)

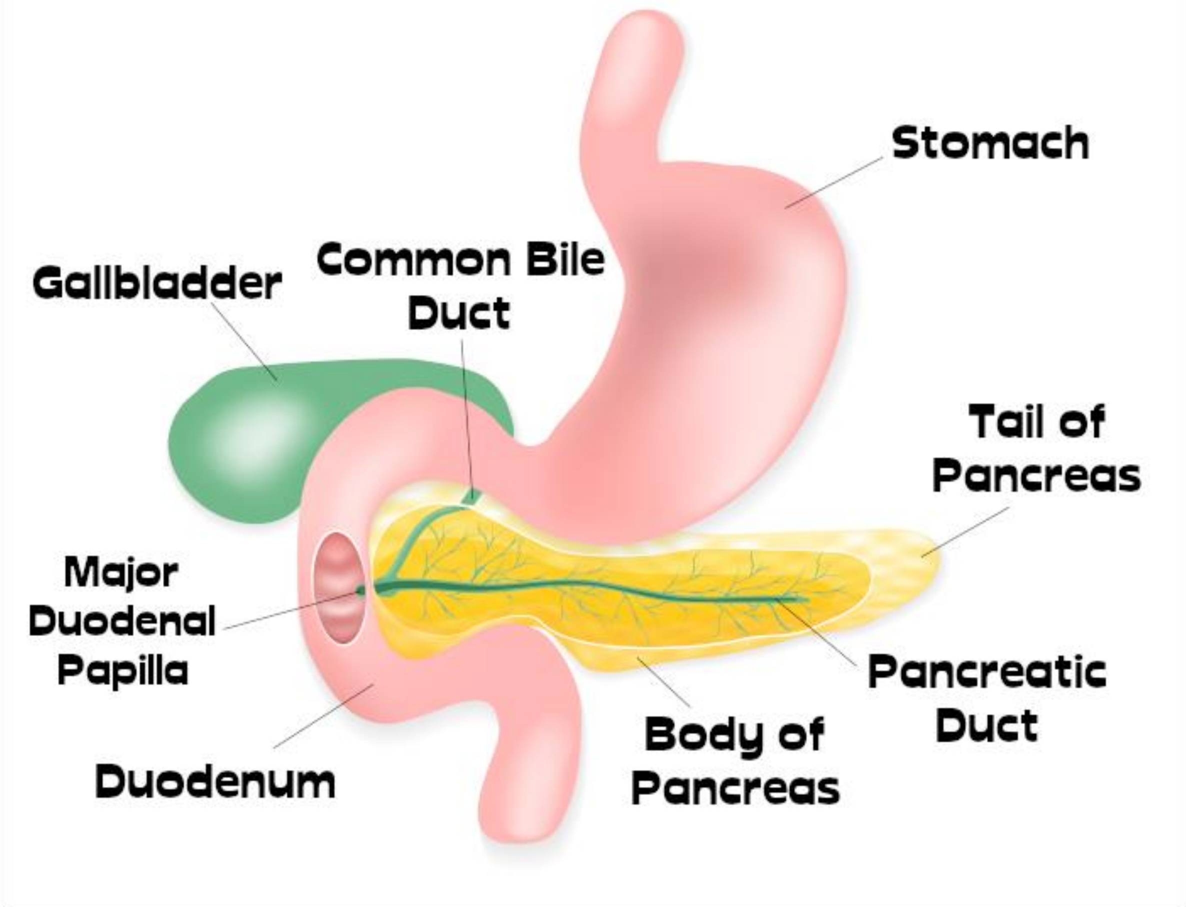

Fig. 2: Diagram of pancreas in relation to the duodenum (www.bling.com/images)

IJNRD
Research Through Innovation

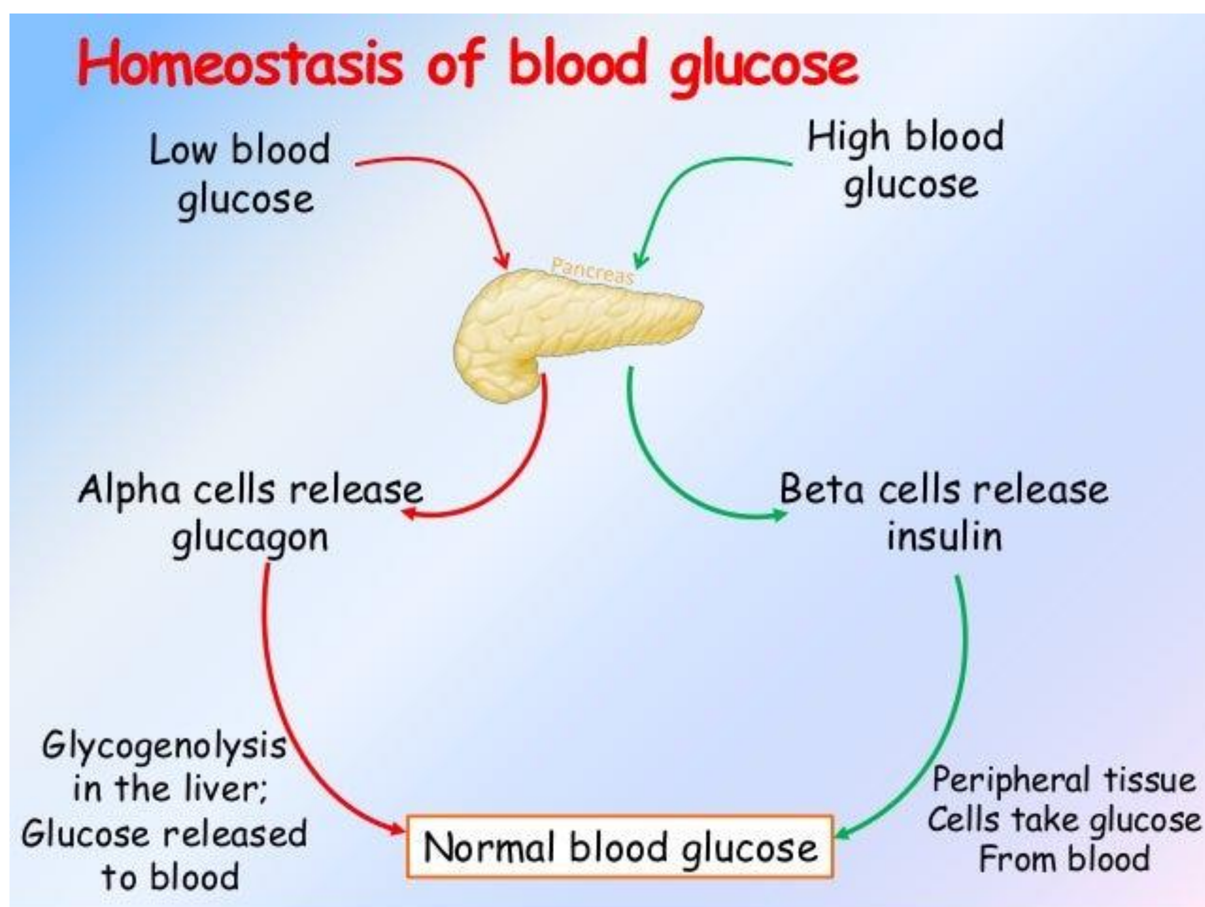

Fig. 3: The regulation of glucose levels through Homeostasis (www.researchgate.net/blood sugar regulation)

MATERIALS AND METHODS

2.1 Cell culture and maintenance: Research materials including Hep G2 cell line, T-75 flasks, Eppendorf tubes, 96-well plates, Bradford reagent test kit, cell culture media (DMEM) and all reagents for buffer preparation were purchased accordingly.

First round of the experiment was conducted to test the viability of the HepG2 cell line, a pinch of the cells was inoculated into T-75 flasks containing liquid media (DMEM) and incubated with shaking for one week, cell adherence and confluence was determined which confirmed the viability of the imported HepG2 cells (10)

Second round of the experiment commenced immediately by preparing fresh culture media, five T-75 flasks containing DMEM supplemented with high glucose and 10% fetal calf serum (FCS), 1% Penicillin and streptomycin were used to culture the HepG2 cells at 37° C for seven days. The cells were passaged using trypsin/EDTA every after 3 days, with fresh culture media under the same condition as described above. At 70% confluence, cells were washed with PBS, treated with trypsin-EDTA and incubated for 5 minutes for cells to completely detached. 10 ml of DMEM was added and centrifuged for 5 minutes, the supernatant was discarded and the pellets were suspended in 6 ml of culture media, 1ml of the suspension was added to 19ml of media to achieve 5% cell concentration. The cells were divided into six 6 well plates and allowed to adhere for 24 hours. After adherence, the cells were treated with high glucose and different concentrations insulin and claramine (11) as presented in the following tables.

Treatment	High glucose	High glucose	High glucose	High glucose	High glucose	High glucose
Glucose	25nM	25nM	25nM	25nM	25nM	25nM
Insulin	0nM	10nM	20nM	50nM	100nM	200nM

Treatment	High glucose	High glucose	High glucose	High glucose	High glucose	High glucose
Glucose	25nM	25nM	25nM	25nM	25nM	25nM
Insulin	50nM	50nM	50nM	50nM	50nM	50nM
Claramine	0µM	0.5µM	1.0µM	2.0µM	4.0µM	10.0µM

Treatment	High glucose	High glucose	High glucose	High glucose	High glucose	High glucose
Glucose	25nM	25nM	25nM	25nM	25nM	25nM
Insulin	100nM	100nM	100nM	100nM	100nM	100nM
Claramine	0µM	0.5µM	1.0µM	2.0µM	4.0µM	10.0µM

Treatment	High glucose	High glucose	High glucose	High glucose	High glucose	High glucose
Glucose	25nM	25nM	25nM	25nM	25nM	25nM
Insulin	0nM	10nM	20nM	50nM	100nM	200nM
Claramine	0µM	0.5µM	1.0µM	2.0µM	4.0µM	10.0µM

The standard glucose solution used was supplied by SIGMA-ALDRICH. 80µL of reagent was mixed with 40 µL of sample in 96-well plate and incubated for 30 min at 37°C, 80µL of 12N H_2SO_4 was added and absorbance of the reaction mixture was measured with Multiscan-FC spectrophotometer (Thermo-Scientific) at 540nm. and the amount of glucose utilized by the cell was calculated and used for protein normalization (10)

2.2 Cell lysis and determination of glucose utilization: The cells in each of the 6 wells were thoroughly washed with about 1mL of PBS, treated with RIPA buffer to break the cells. The broken cells were scraped into 6 Eppendorf tubes and centrifuged at 7000rpm for 1min at 4°C, the supernatant which contains the cell extract were transferred each into an Eppendorf tube making a total of 6 different tubes, and preserved in the -20°C refrigerator for glucose and protein assays (11)

2.3 Glucose and Enzyme Assays: The standard glucose solution used was supplied by SIGMA-ALDRICH. 80µL of reagent was mixed with 40 µL of sample in 96-well plate and incubated for 30 min at 37°C, 80µL of 12N H_2SO_4 was

added and absorbance of the reaction mixture was measured with Multiscan-FC spectrophotometer (Thermo-Scientific) at 540nm and the amount of glucose utilized by the cell was calculated and used for protein normalization

Bradford assay was used to estimate the concentration of protein in intracellular the reaction samples. Standard protein concentrations of 0µg/ml, 31µg/ml, 63µg/ml, 125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml were prepared to establish a standard curve. The experiment was performed in triplicate of the various standards and 1:20 dilution of the cell lysate in 96 well plate. 5µL of each of the cell lysates were diluted in 95µL ddH_2O in an Eppendorf tube, from which 10ul of each were added in 3 wells of the 96-well plate. 200uL Bradford reagent was added into each well, absorbance was read immediately by Multiscan-FC spectrophotometer (Thermo-Scientific) at 595nm (11)

RESULTS AND DISCUSSION

Results

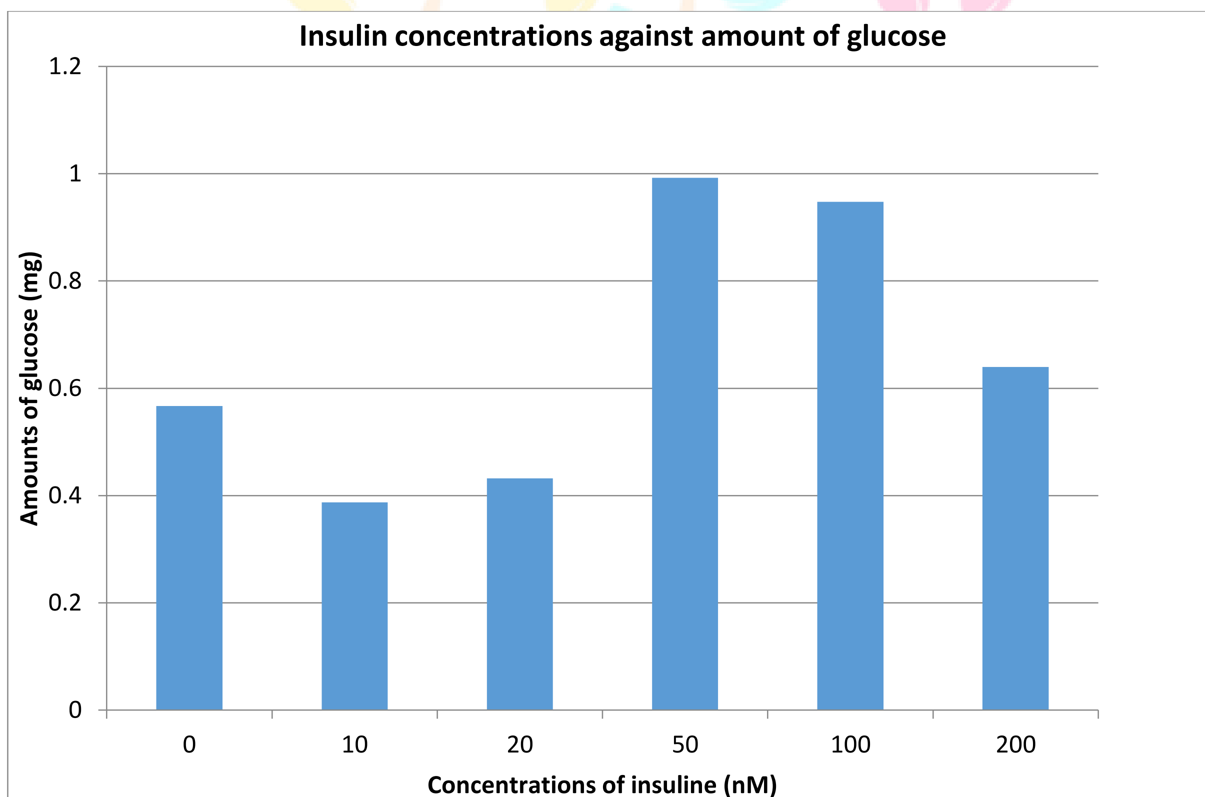

Fig. 4. A chat showing the effects of insulin on glucose uptake in Hepg2 cells.

Effects of different doses of insulin on glucose uptake in HepG2 cells was determined, results were analysed and presented as column chat in figure 1 above. The results showed optimum insulin action at 50nM concentration. Glucose uptake initially decreased to about 0.4 mg at 10nM concentration of insulin, then increased to 1mg maximum at 50nM and gradually decreased to 0.6nM at 200nM concentrations of insulin.

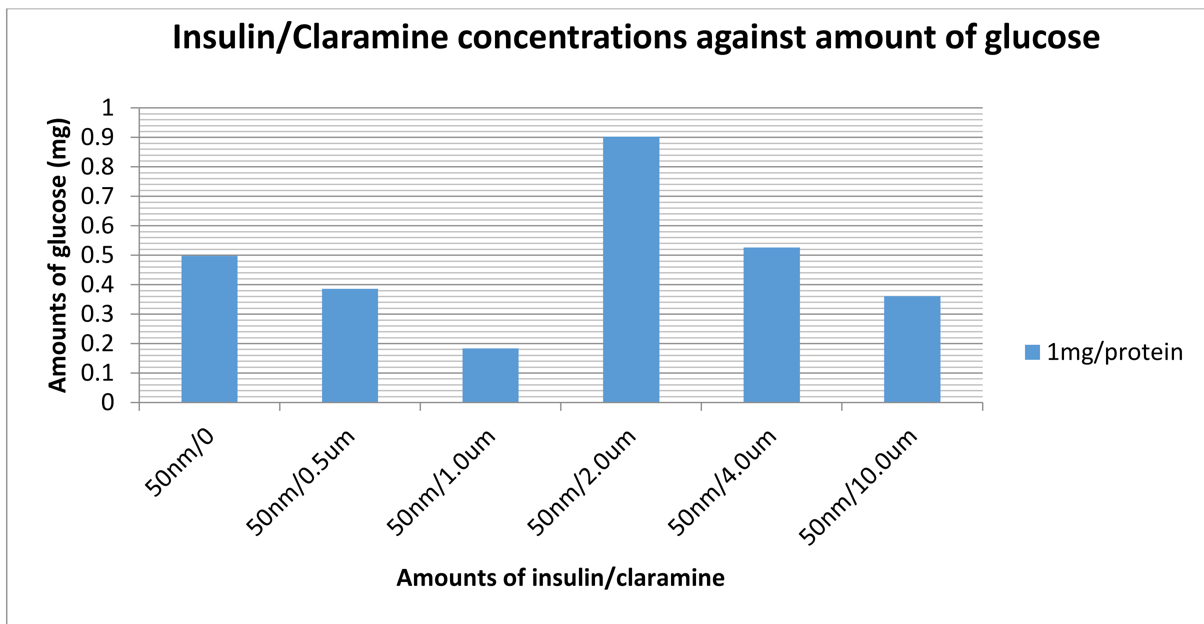

Fig 5. A chart showing the relationship between insulin/claramine and enzyme protein expressed by the cells.

Combination of 50nM insulin with various concentrations of claramine shows trend in the uptake of glucose by HepG2 cells, glucose uptake decreased from 0.5 mg to 0.18 mg with increase in claramine concentration from zero to 1.0µM however, glucose uptake increased to highest 0.9mg at 2.0 µM concentration and later decreased to about 0.4 mg at 10 µM claramine concentration. The following chat shows the combined effects of 100nM insulin concentration and various concentrations of claramine were determined on glucose uptake in HepG2 cells.

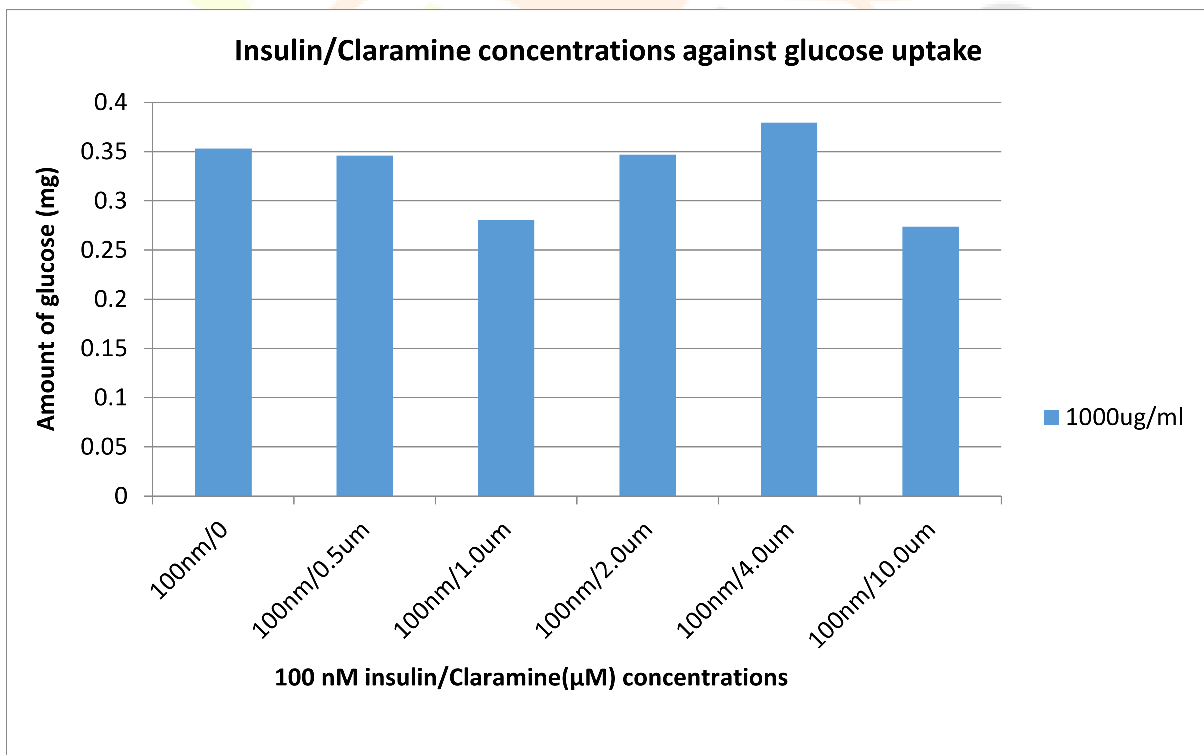

Fig 6. Showing the column chart of insulin/claramine concentrations against glucose uptake

The chat in figure 3 above shows relatively little and irregular variation in glucose uptake by HepG2 cells at 100nM insulin with different concentrations of claramine.

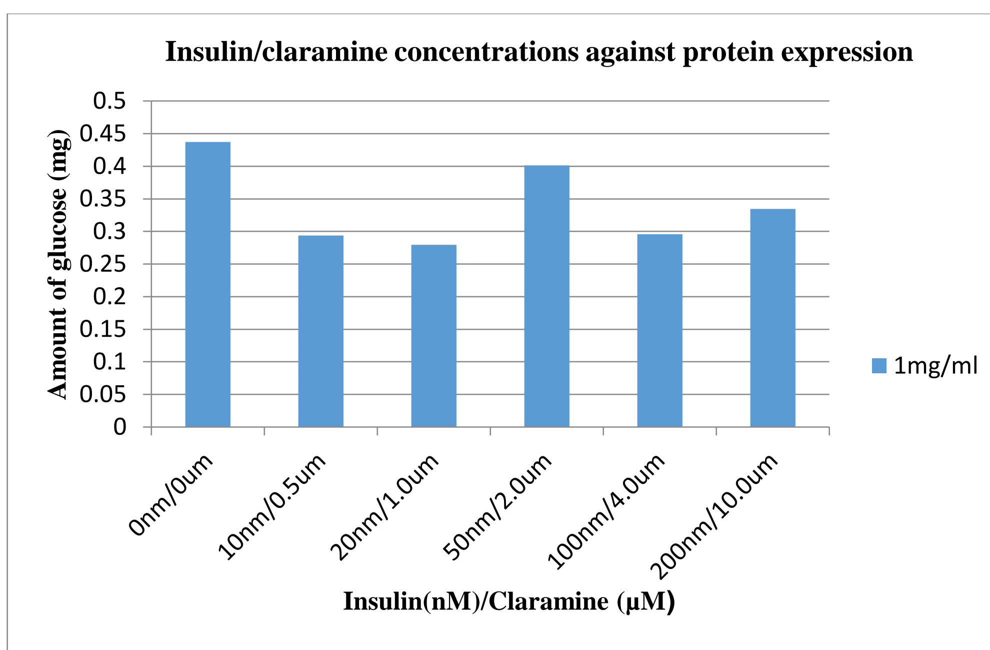

Fig 7. Showing the amount of glucose utilization with respect to joint variation of insulin and claramine concentrations

Discussion

Results shows the glucose uptake with respect to change in both insulin and claramine concentrations, the uptake of glucose decreased with the joint variation of insulin and claramine from 0.44mg to 0.30mg at 20nM insulin and 1μM claramine, this however increased to 0.40mg at 50nM insulin and then irregularly decreased to 0.34mg at 200nM insulin and 10μM claramine concentration. The findings in this study is in line with other researchers who discovered that claramine and others like Trodusquinine are capable of activating insulin signalling thereby promoting glucose metabolism (4,5,8, 10, 12).

Conclusion

Hepatoma cell line (HepG2) can model the hepatocyte for insulin action and claramine can be employed as an alternative more easily manufactured non-cytotoxic compound for treatment of type II diabetes

ACKNOWLEDGEMENT

This research work was sponsored by Tertiary Education Trust Fund (TETfund) Abuja. Appreciation goes to Technical staff in the Nnamdi Azikiwe University, Awka Anambra state, Nigeria for Laboratory assistance.

REFERENCES

[1]Waugh, A. and Grant, A. (2010). Ross and Wilson Anatomy and Physiology in Health and Illness. 11th Ed.

[2]Satyanarayana, U. and Chakrapani (2018). Biochemistry (with Biomedical Concepts, Clinical Correlates and Case Studies. Elsevier Books and Allied, 5th Ed

[3] Soyoye, D.O., Ikem, R.T., Kolawole, B.A.,Oluwadiya, K.S., Bolarinwa, R.A. and Adebayo, O.J. (2016). Prevalence and Correlates of Pheripheral Arterial Disease in Nigerians with Type 2 Diabetes

[4]Aji, G., Huang, Y., Ng, M.L., Wang, W., Lan, T., Li, M., Li, Y.. Chen, Q., Li. R. Yan, S., Tran, C., Burchfield, J.G., Couttas, T.A., Cherr, J. Chung, H., Liu D., Wadham, C., Hogg, P.J., Gao, X., Vadas, M.,

Gambiae, J.R., Don, A.S., Xia, P. and Qi, Y. (2020). Regulation of Hepatic insulin signalling and glucose homeostasis by sphingosine kinase 2. *PMAS* http://www.pmas.org. vol.117, no 39

[5]Lewis, G.F., Carpentier. C.A., Pereira, S.Hahn, M. and Glacca, A. (2021). Direct and Indirect Control of Hepatic Glucose production by insulin. *Cell metabolism*. 33, April 6

[6]Sun, J., Qu, C., Wang, Y., Hung, H., Zhang, M., Zou, W. (2016). Type 2 diabetes mellitus and protein-tyrosine phosphate 1b. Journal of Diabetes, Metabolic Disorders and Control. 3(8): 180 – 183.

[7] Roberts, M.B.V. (1983). Biology; A functional Approach. Buttler and Tanner Ltd. UK

[8]Gaborit, B. Govers, R., Attie, A., Brunei, M.J., Morange, P. and Peiretti, F. (2021). *Journal of Biological Chemistry* Research Article, 297(1) 100818

[9]Okechukwu, C. (2023). 6 Essential Things Every Diabetic Should Know. htps:healthcoachokechukwu.com.ng/

[10]Molinaro, A., Becattin, B. and Solinas, G. (2020). Insulin Signalling and Glucose metabolism in different Hepatoma cell lines deviate from Hepatocyte Physiology toward a Convergent Aberrant Phenotype. *Scientific Reports- Nature Research*, 20:122031

[11]Vidiya, V. L., Yang, H. Lerapetritou, M.G., Roth, M.C. (2010). Effects of Glucose and Insulin on HepG2—C3A Cell metabolism. *Biotechnology Bioengineering* Oct 1, 107(2):347- 56

[12]Zhaohong, Q., Pandey, N.R., Zhou, X., Stewart, C.A., Hari, A., Huang, H., Stewart, A.F., Brunei, J.M and Chen, H. (2015). Functional properties of Claramine; A novel PTP1B Inhibitor and Insulin-mimetic compound. *Biochemical and Biophysical Research Communications*. Vol. 455, issue 1, pp 21-27

Web References (Accessed 15/08/2023)

https://www.who.int/health-topics/diabetes

https://www.who.int/news-room/fact-sheets/details/diabetes

https://www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/

https://www.cdc.gov/diabetes/basics/diabetes/

https://en.wikipedia.org/wiki/diabetes

https;//www.bing.com/images