International Journal of Modern Science and Technology, 2018;3(10):208-212.
ISSN: 2456-0235.
www.ijmst.co

Research Article

Diabetes Induction with Streptozotocin and Insulin Action on Blood Glucose Levels in Albino Rats

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Abstract

The present study was designed to investigate diabetes induction with streptozotocin (STZ) in albino rats and the effect of insulin on the blood glucose levels of rats induced with diabetes. Fasting blood sugar (FBS) and oral glucose tolerance test (OGTT) was used to determine glucose levels in experimental groups for 14 days. The results of FBS on days 0, 7 and 14 show that rats treated with 4 µl/kg of body weight daily maintain normal glucose levels (99.50±3.51, 95.83±5.19 and 96.83±5.04) as those which were not induced with diabetes (89.33±5.32, 89.17±4.26 and 89.17±5.19), while the none treated rats were h diabetic all through (375.33±39.53, 381.67±35.44 and 375.67±36.74). OGTT at day 14 showed initial rise in glucose levels at 0 min for non-diabetic and insulin treated diabetic rats after administration of 2 g/kg body weight load of glucose, at 60-120 min normal levels were maintained (100.67±9.1, 117.00±19.0, 95.83±7.3 and 91.83±9.1, 101.00±13.4, 91.67±10.0) respectively. Conversely non treated rats were unable to achieve normal levels (287.00±19.8, 360.83±40.8 and 293.50±13.5). This finding may be helpful in diabetes research that has to do with regulation and maintenance of glucose levels.

Keywords: Diabetes; Insulin; Glucose; Strptozotocin.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. There are three broad categories: type 1, type 2, and gestational diabetes. Although there are other types that are specific to other individual causes [2]. Of these categories the most prevalent of them is the type 2 diabetes having 90% of all diabetes cases at global level, as reported by International Diabetes Federation [3]. Recent estimates by World Health Organization (WHO) indicate that, there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 if no action is taken [4]. However, data from the International Diabetes Federation (IDF) revealed that the WHO estimates has already been reached in 2011. The IDF expects an even higher number of 552 million affected persons in 2030 [5, 6]. This increase in prevalence is expected to be more in developing countries, the Middle Eastern crescent, Indian sub-continent and Sub-Saharan Africa where the prevalence rate up to 13% have been recorded [7].

Several pathogenic processes are involved in the development of DM. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently co-exist in the same patient, and it is often unclear which abnormality is the primary cause of the hyperglycemia [1].

Symptoms of DM include hyperglycemia, polyuria, polydipsia, weight loss, sometimes with polyphagia, blurred vision autonomic and peripheral neuropathy which causes charcot joints and foot ulcers. Impairment of growth and susceptibility to certain infections

Received: 28.09.2018; Received after Revision: 13.10.2018; Accepted: 14.10.2018; Published: 17.10.2018

may also accompany chronic DM [3, 8]. The disease progression in neuropathy which may lead to loss of sensory perception is clinically characterized by the development of vascular abnormalities, such as capillary basement membrane thickening and endothelial hyperplasia with subsequent diminishment in oxygen tension and hypoxia [8, 9].

Insulin has been identified and used in the management of diabetes in humans for several years. However, the search for permanent solution to diabetes is till at the forefront of research in science and medicine. To this course, animal models have provided suitable means for diabetes experimentation that would be impossible in humans [10]. Animals used in diabetic experiments are of varied species. However, rodents have been reported to be the most used and extensively studied experimental animals in induction of DM [11]. The type of induction and model animal of choice is often the researcher's decision to make, and greatly depends on the nature of the research. Factors that has influenced the use of either Spontaneous/genetically derived diabetic animals, diet/nutrition induced diabetic animals, chemically induced diabetic animals, surgical diabetic animals or Transgenic/knock-out diabetic animals as a model may include: Type of diabetes, duration of the research, cost of induction, skills/experience of the researcher and a host of other factors [12]. The advantages of STZ being relatively cheaper and easier to develop and maintain, reliable and very effective, and ability to be used for longer experimental study are reasons for its frequent use. Knowledge of insulin activity in STZ induced diabetes will provide good information for albino rat diabetes studies centred on blood glucose regulation with insulin. This study seeks to establish the hypoglycemic activity of insulin in STZ induced diabetes in albino rats.

Materials and methods

Experimental animals

Care of animals was in line with Institute of Health Guide for Care and Use of Laboartory Animals [13]. Albino wistar rats weighing 160-250 g were allowed to acclimatize for 7 days prior to the initiation of the experiment in plastic cages and under laboratory conditions (temperature 22±2°C, relative humidity 60-70%, and 12 hr-12 hr light-dark cycle). Animals were fed with balanced diet purchased from UAC foods Nigeria Ltd. and water *ad libitum*.

Experimental design

Three groups of animals (n=6) was used to establish diabetes induction by STZ and hypoglycaemic activity of insulin in induced rats for a period of 14 days after acclimatization period. Group 1 =control (none diabetic), group 2 = diabetic (none treated) and group 3 = diabetic (insulin treated). Fasting blood sugar (FBS) was done on days 0, 7 and 14 and oral glucose tolerance test (OGTT) on the 14th day at 20, 40, 60, and 120 min.

Induction of diabetes Mellitus and insulin administration

The method employed by [11] was used. After a 12 hr fast, rats received a single intra-peritoneal injection of 65 mg/kg STZ (Caymanchem) freshly prepared in 0.1M sodium citrate buffer (JHD® Ltd) of pH 4.5. At 8 days after STZ injection, blood glucose measurement was performed on tail-vein blood with a glucometer (Accu-Chek® S.No.Gb10082701). Rats whose glucose tolerance test and fasting blood glucose levels exceeded 250 mg/dL (13.9 mmol/dL) were considered diabetic [11]. Water intake and weight was monitored throughout the study. 4 µL/Kg body weight of insulin injection (Wockhardt Ltd. India) was administered to rats 30 min before OGTT and daily in FSB studies.

Statistical analysis

The results were analysed statistically by Tukey post hoc test using SPSS software version 21. Results were expressed as mean ± S.D and considered statistically significant at p value <0.05.

Results and discussion

In table 1, the results of fasting blood sugar for STZ induced diabetic and non-diabetic albino rats are presented. They show that, They show that the sugar levels of STZ-induced diabetic rat group that was not treated was above normal levels (80-140 mg/dl), while both the non-diabetic and insulin treated diabetic group were within normal levels. There was a significant difference ($p<0.05$) in the sugar levels of non-treated diabetic rats (375.33±39, 381.67±35.44 and 375.67±36.74) when compared with the non-diabetic control group (89.33±5.32, 89.17±4.26 and 89.17±5.19) and

the diabetic group treated with insulin (99.50±3.51, 95.83±5.19 and 96.83±5.04) for day 0, 7, and 14 respectively. Conversely, the non-diabetic group and diabetic group treated with insulin showed no significant difference ($p<0.05$). These results demonstrate that insulin has a blood glucose lowering effect on STZ-induced diabetes in albino rats and that the administration of STZ induced diabetes in the animals.

Table 1. Fasting Blood Sugar (FBS) in Diabetic and non-Diabetic Albino Rats to indicate Diabetes Inducing Effect of Streptozotocin (STZ) in Albino Rats

Day	Groups		
	CND (mg/dl)	DNI (mg/dl)	DIT (mg/dl)
0	89.33±5.32[a]	375.33±39.53[b]*	99.50±3.51[a]
7	89.17±4.26[a]	381.67±35.44[b]*	95.83±5.19[a]
14	89.17±5.19[a]	375.67±36.74[b]*	96.83±5.04[a]

CND= Control non diabetic, DNT= Diabetic (no Insulin), DIT= Diabetic (insulin treatment)
*=significant difference ($p<0.05$), alphabets = Homogeneous subset

This is indicated by the glucose levels within normal range (70-140 Mg/dl) presented by rats which were not administered STZ and those which were administered STZ but treated with insulin. These findings are in agreement with that of [14, 15]**.** Hyperglycemia observed I the diabetic group not treated with insulin was the effect of selective pancreatic beta cell toxicity of STZ which causes cell death [16, 17]. Carbamoylation and alkylation of cellular components, release of nitric oxide (NO), free radical generation and Oxidative stress, and inhibition of O-GlcNAcase are proposed mechanisms for STZ action [17].

Table 2 presents the results of oral glucose tolerance test for STZ induced diabetic and non-diabetic albino rats. They show that the sugar levels of STZ-induced diabetic rat group which was not treated was above normal levels (80-140mg/dl), while both the non-diabetic and insulin treated diabetic group were within normal levels. A significant difference ($p<0.05$) was observed in the sugar levels of non-treated diabetic rats (287.00±19.8, 469.00±37.9, 410.50±41.1, 360.83±40.8 and 293.50±13.5) when compared with the non-diabetic control group (100.83±9.1, 277.17±21.3, 177.00±19.0 and 95.83±7.3) and the diabetic group treated with insulin (91.83±9.1, 294.33±22.4, 173.17±10.2, 101.00±13.4 and 91.67±10.0) for 0, 20, 40, 60 and 120 min respectively. There was a significant ($p<0.05$) difference in the blood glucose levels of the non-diabetic rats and the diabetic rats that were treated with insulin when compared to the diabetic rats that was not treated.

Table 2. Oral Glucose Tolerance Test (OGTT) in Diabetic and non-Diabetic Albino Rats to Confirm Diabetes Inducing Effect of Streptozotocin (STZ) in Albino Rats

Day	Groups		
	CND (mg/dl)	DNI (mg/dl)	DIT (mg/dl)
0	100.67±9.1[a]	287.00±19.8[b]*	91.83±9.1[a]
20	277.17±20.2[a]	469.00±37.9[b]*	294.33±22.4[a]
40	210.83±21.3[a]	410.50±41.1[b]*	173.17±10.2[a]
60	117.00±19.0[a]	360.83±40.8[b]*	101.00±13.4[a]
120	95.83±7.3[a]	293.50±13.5[b]*	91.67±10.0[a]

CND= Control non diabetic, DNT= Diabetic (no Insulin), DIT= Diabetic (insulin treatment)
*=significant difference ($p<0.05$), alphabets = Homogeneous subset

In figure 1, the curve of oral glucose tolerance test is presented. The results show that all the experimental animals had a rise in the glucose levels upon administration of the glucose as observed at 20min. this further decreased through the course of time and maintained normal levels between 60-120 min for both non diabetic groups and the diabetic group that was treated with insulin. Conversely, the rise in glucose levels for the non-treated diabetic rats dropped but did not reach normoglycemic levels. These finding confirms the inability of the non-treated diabetic rats to tolerate glucose and the glucose lowering effect of insulin in diabetes induced by STZ.

The OGTT of rats administered STZ increased beyond normal level (140 mg/dl). The rats which were not induced with diabetes and those which were induced but treated with insulin presented glucose levels within normal range (70-140 mg/dl), similar findings have reported [18, 19]. The reduced glucose levels in the diabetic rats treated with insulin is the result of insulin's binding to cell receptors thereby making glucose available for utilisation by cell. This is achieved through binding of insulin's alpha subunits to the membranes subunits leading to auto phosphorylation of the two beta subunits that extends to the cytoplasm by having bonds of the two subunits, and induces the conversion of active protein kinase through

intracellular signalling cascade [20]. This demonstrates that insulin lowers the glucose levels of STZ-induced diabetes and that diabetic rats are incapable of tolerating glucose.

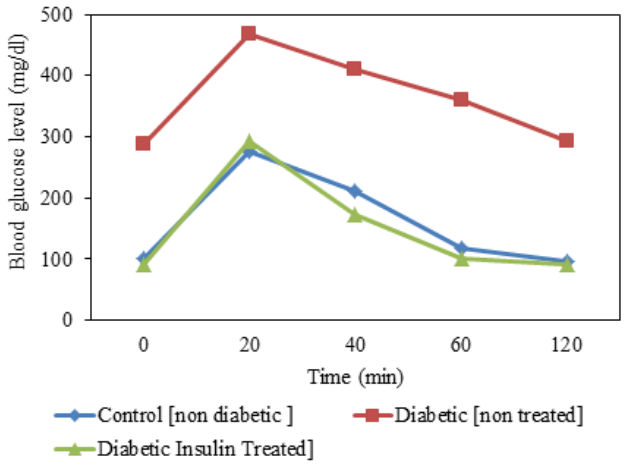

Figure 1. Oral Glucose Tolerance Test (OGTT) Curve of Diabetic and non-Diabetic Albino Rats to Confirm Diabetes Inducing Effect of Streptozotocin (STZ) in Albino Rats

Conclusions

The present work attempted to study the induction of diabetes with single dose administration of streptozotocin and the action of insulin on the blood glucose levels of albino rats induced with diabetes for a period of 14 days. The results obtained show that insulin lowers the glucose level of streptozotocin induced diabetes in rats and may be used as a standard for both long and short term research that has to do with the regulation and maintenance of glucose levels in albino rats. Such researches are centred on diabetes complications and nutritional management for diabetics and test for new drugs.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank Mr Vincent Upev of Biochemistry Department, College of Veterinary Medicine, University Agriculture Makurdi for assistance with Streptozotocin.

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