DOI: 10.7860/JCDR/2021/46729.15567

Original Article

Animal Research Section

Effective Dose of Streptozotocin to Induce Diabetes Mellitus and Variation of Biophysical and Biochemical Parameters in Albino Wistar Rats

SUHASINI PADUGUPATI[1], S RAMAMOORTHY[2], KUMAR THANGAVELU[3], DVHS SARMA[4], DEEPAK JAMADAR[5]

ABSTRACT

Introduction: There is a need to develop diabetic animal model, to have a better understanding of the complications of diabetes mellitus. The dose of Streptozotocin (STZ) to induce diabetes mellitus in animals is important as it may lead to inadequate induction of diabetes or mortality. Intravenous injection of STZ in adult Wistar rats, leads to the degeneration in Langerhans islet β-cells and induces experimental diabetes mellitus in 3-5 days.

Aim: To optimise the dose of STZ to create a diabetic animal model with sustained hyperglycaemia and to compare the changes in body weight, serum glucose and C-peptide levels between non diabetic and diabetic rats.

Materials and Methods: This experimental animal study was conducted at animal house, Pal amur Bioscience Pvt., Ltd. The sample size included 30 albino Wistar rats divided into five groups T0, T1, T2, T3 and T4 with six rats in each group (three males and three females). Group T0 was the control, while STZ at different concentrations were administered intraperitoneally in group T1, T2, T3 and T4, respectively. Blood samples were drawn from retro-orbital plexus of animals and blood glucose, C-peptide levels along with the body weight was checked on 7th, 14th, 21st and 28th day. The F statistics, one-way Analysis of variance (ANOVA) was used to compare the different groups. Denny's test was used to compare the control group versus different test groups.

Results: When compared with the control group T0 on 7th, 14th, 21st and 28th day, the test group T1 had no variation in the body weight. On the other hand groups T2, T3 and T4 had variations in the body weights. Initially there was increase in the weight, later here was a gradual decrease in the body weight when compared to the control group. Hyperglycaemic profile (blood glucose level >120 mg/dL) was achieved in group T1, T2, T3 and T4 after 7 days. High mortality rate was observed in group T4 followed by group T3. Group T2 had persistent hyperglycaemia while group T1 had reversible hyperglycaemic profile. The C-peptide levels were gradually decreased in the test groups and it was statistically significant (p-value <0.0001).

Conclusion: Intraperitoneal dose of STZ of 55 mg/kg created diabetic animal model with persistent hyperglycaemia. However, dose above increased the mortality rate and below failed to create diabetic animal model.

Keywords: Blood glucose, Body weight, C-peptide, Diabetic animal model, Persistent hyperglycaemia

INTRODUCTION

Diabetes mellitus is often called 'the silent killer', as there are no serious symptoms till the serious complication arises, effecting the major organs in the body. The metabolism of carbohydrates, fats, proteins and electrolytes are affected by several chronic disorders leading to various complications that can be classified into acute, subacute and chronic [1]. Acute complications hypoglycaemia, diabetic ketoacidosis, hyperosmolar and hyperglycaemic non ketotic syndrome are acute complications of the chronic metabolic disorders [2], while subacute complications include thirst, polyuria, lack of energy, visual blurriness and weight loss [3]. Chronic hyperglycaemia causes glycation of body proteins which in turn leads to complications that may affect the eyes, kidneys, nerves and arteries [4]. The prevalence of diabetes mellitus is increasing yearly among human beings and different drugs are being used to treat the disorder. The prevalence of diabetes in the world among adults (aged 20-79 years) was 6.4%, affecting 285 million adults, in 2010. Between 2010 and 2030, there will be a 69% increase in numbers of diabetics in developing countries and a 20% increase in developed countries leading to increase by 7.7%, 439 million adults by 2030 [5]. To understand severity and complications of disease and to analyse effects of drugs in diabetes mellitus it is necessary to create diabetic animal models. From decades diabetic animal models have been used to understand the disease process and to understand the pharmacokinetics and pharmacodynamics of existing and newly developed drugs [6]. Various methods are being used in laboratories to create diabetic animal models, including surgical method such as pancreatectomy and pharmacological methods such as by alloxan monohydrate and streptozotocin. Use of streptozotocin was considered predominantly as a diabetogenic agent [7,8]. The study helps to establish a dose of streptozotocin to create diabetic animal model with persistent hyperglycaemia, so that adverse effects could be studied and the prevention strategies can be formulated effectively.

Streptozotocin (STZ) is an anti-neoplastic, antibiotic used for cancer chemotherapies [9,10]. It is {2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose)} a naturally occurring compound, produced by the soil bacterium streptomyces a chromogens that exhibits broad spectrum of antibacterial properties [11]. It is a mixture of α- and β-stereoisomers that appear as pale yellow or off-white crystalline powder. The STZ with a molecular formula C8H15N307 with a molecular weight 265 gm/mol [11]. The STZ is a cytotoxic glucose analogue. It inhibits Deoxyribonucleic Acid (DNA) synthesis in bacterial and mammalian cells. The STZ is toxic to pancreatic βcells [12].

Although the mechanism of action is not clear but it is thought that STZ causes death of pancreatic β-cells by DNA alkylation and

produces diabetes mellitus in experimental animals by entering in β-cells through cell membrane of GLUT2 glucose transporter [13,14]. one study was conducted to induce diabetes mellitus in rats, a large dose (i.e., 100 mg/kg) of STZ [15], few studies support a moderate dose (i.e., 60 mg/kg) and other researchers proved that a small dose (i.e., 40 mg/kg) of the drug can induce diabetes in rats [16,17]. The STZ is an expensive drug for creating a diabetic animal model, still preferred over alloxan 14 mmol. To create a diabetic animal model, one should have a thorough knowledge of dose of STZ at which he may achieve a sustained diabetic profile in animal models with less mortality rate. The connecting peptide (C-peptide) is 31 aminoacid polypeptide that connects A and B chain in proinsulin. Pre proinsulin is translocated to the β-cells of pancreas with A chain, C-peptide and B chain. Signal sequence is cleaved leading to formation of proinsulin. Now this proinsulin is cleaved in the golgi apparatus, forming insulin molecule [18-20]. The C-peptide is a useful and widely used method of assessing pancreatic beta cell function [21,22].

The objective of the present study was to optimise the dose of STZ to create a diabetic animal model, to document the toxic dose and to compare the changes in body weight, serum glucose and C-peptide levels between normal and diabetic rats.

MATERIALS AND METHODS

It was an animal based experimental study conducted at Animal House of Faculty of Palamur Bioscience Private Limited for a period of one month (June 2018). The study was approved by Institutional Animal Ethics Committee (IAEC) Palamur Biosciences Private Limited (CPCSEA Registration Number -1312/PO/ReBiBt-S/09/CPCSEA). Animals were obtained from in house bred at Palamur Biosciences Pvt., Ltd.

Sample size: The sample size included total 30 albino Wistar rats weighing between 120-200 gm. These were divided in to five groups (T0, T1, T2, T3 and T4). Each group included six rats. Each group included three male and three female rats. All the animals were fed by standard rat pellet diet and were allowed for free access to water. The rats were housed in standard cages at a constant temperature (15°–25° C) with fixed 12:12 hour light-dark cycle.

Rats in all the groups were subjected to overnight fasting. Next day the rats in control group (T0) were subjected to intraperitoneal fresh normal saline (2 mL). Group T1, T2, T3 and T4 were the test group. Next day induction of diabetes mellitus was performed at different concentrations of STZ among T1,T2,T3 and T4 groups respectively with the doses of 45 mg/kg, 55 mg/kg, 65 mg/kg and 75 mg/kg intraperitoneally in one shot.

The STZ before use was dissolved in 0.1 M of freshly prepared sodium citrate buffer, pH 4.5, made isotonic by the addition of 0.25 M NaCl. Glucose water of 5% was given for two days to prevent drug induced hypoglycaemic shock. Body weight of the rats, blood glucose levels and C-peptide levels were noted after 7th days, 14th days, 21st days and 28th days of STZ injection. The blood sample was collected from retro-orbital plexus and centrifuged at 1000 RPM (Revolutions Per Minute) for 20 minutes and the serum (supernatant) was collected.

Blood glucose: It was estimated by semi-auto analyser using ERBA blood glucose kit. Method used was Trinder's method [23]. Standard procedure as per the instruction manual was followed. Principle of this method was glucose in sample is oxidised to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyses the oxidative coupling of 4-aminoantipyrine with phenol to yield coloured quinonimine complex, with absorbance proportional to the concentration of glucose in sample.

C-peptide levels (C-P): It was estimated by Enzyme-linked Immunosorbent Assay (ELISA) test kit with catalogue number ITER0304 in an ELISA reader with the detection range of 0.07-5 ng/mL. Standard procedure as per the instruction manual was followed. Principle of this assay was based on sandwich ELISA technology [24]. The C-peptide antibody was precoated onto 96-well plates and the biotin conjugated anti C-peptide antibody.

The standards, test samples and biotin conjugated detection antibody was added to the wells subsequently, and washed with wash buffer. Horseradish peroxidase (HRP)-Streptavidin was added and unbound conjugates were washed away with wash buffer. The TMB substrates were added. 3,3′,5,5′-Tetramethylbenzidine or TMB was catalysed by HRP to produce a blue colour product that changed to yellow after adding acidic stop solution. The density of yellow was proportional to the C-peptide amount of sample captured in plate. Read the OD absorbance at 450 mm in a microplate reader, and then the concentration of C-peptide was measured.

STATISTICAL ANALYSIS

The data has been entered in the excel sheet, column wise and row wise. After entering, the data has been analysed with Graph pad prism software version 6.3.1. The F statistics, one-way Analysis of Variance (ANOVA) was used to compare the different groups. Denny's test was used to compare the control group versus different test groups. Statistical significance was tested for p-value <0.05.

RESULTS

The overall group analysis of body weights of all rats in different groups at variable time intervals were analysed with F statistics. The F-value of over group analysis of body weights of rats in different groups on 28th day after STZ was 4.962 with p-value 0.0044. This was represented in [Table/Fig-1]. To conclude there was a significant difference (decrease) in the body weight between the control group T0 versus test group T3 and T4 on 28th day after injection of STZ.

The overall group analysis of blood glucose levels of all rats in different groups at variable time intervals were analysed with F statistics. The F-value of over group analysis of blood glucose levels of rats in different groups on 7th day after STZ was 4.369 with p-value=0.0081. The F-value of over group analysis of blood glucose of rats in different groups on 14th day after STZ was 0.7327 with p-value=0.5783. The F-value of over group analysis of blood glucose of rats in different groups on 21st day after STZ was 0.8841 with p-value=0.4877. The F-value of over group analysis of blood glucose of rats in different groups on 28th day after STZ was 0.4064 with p-value=0.8023. This was represented in [Table/Fig-2]. To conclude there was a significant difference (increase) in the blood glucose levels between the control group T0 versus test group T3 and T4 on 7th day after injection of STZ.

The overall group analysis of C-peptide levels of all rats in different groups at variable time intervals were analysed with F-statistics. The F-value of over group analysis of C-peptide levels of rats in different groups on 7th day after STZ was 67.54 with p-value <0.0001. The F-value of over group analysis of C-peptide levels of rats in different groups on 14th day after STZ was 59.62 with p-value <0.0001. The F-value of over group analysis of C-peptide levels of rats in different groups on 21st day after STZ was 63.06 with p-value <0.0001. The F-value of over group analysis of body weights of rats in different groups on 28th day after STZ was 86.31 with p-value <0. 0001. This was represented in [Table/Fig-3]. To conclude there was a significant difference (increase) in the C-peptide levels between the control group T0 versus test group T1, T2, T3 and T4 on 7th, 14th, 21st and 28th day after injection of STZ.

Groups	Days							
	7th day		14th day		21st day		28th day	
	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value
Body weight								
T0 vs T1	147.67±11.77 vs 150.00±5.621	0.9998	154.67±12.40 vs 152.17±6.274	0.9998	160.33±13.86 vs 154.83±5.845	0.9972	169.50±13.60 vs 159.50±6.156	0.9884
T0 vs T2	147.67±11.77 vs 155.67±4.926	0.6989	154.67±12.40 vs 154.00±5.933	0.9999	160.33±13.86 vs 149.67±4.633	0.9676	169.50±13.60 vs 143.83±4.401	0.7595
T0 vs T3	147.67±11.77 vs 152.67±8.477	0.9946	154.67±12.40 vs 124.50±61.500	0.9976	160.33±13.86 vs 121.00±59.545	0.2486	169.50±13.60 vs 94.00±72.955	0.0374*
T0 vs T4	147.67±11.77 vs 127.67±62.813	0.5820	154.67±12.40 vs 100.67±78.235	0.0567	160.33±13.86 vs 120.50±59.288	0.2392	169.50±13.60 vs 69.50±76.372	0.0046*
Over all group analysis T0 vs T1, T2,T3 and T4								
	Statistical test with test-value (ANVOA) F-value		p-value					
Day 7	0.8727		0.4947					
Day 14	2.428		0.0756					
Day 21	1.498		0.2328					
Day 28	4.962		0.0044*					

[Table/Fig-1]: Mean variations in body weights (gm) and statistical analysis after the dose of Streptozotocin (STZ).
*p-value <0.05 was statistically significant

Groups	Days							
	7th day		14th day		21st day		28th day	
	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value	Mean±SD	p-value
Blood glucose								
T0 vs T1	88.17±6.11 vs 88.83±7.055	0.9998	86.17±4.66 vs 97.33±8.091	0.9858	89.67±3.93 vs 97.00±10.198	0.9982	89.67±2.94 vs 103.50±11.149	0.9868
T0 vs T2	88.17±6.11 vs 119.33±2.160	0.2599	86.17±4.66 vs 126.33±3.204	0.4596	89.67±3.93 vs 129.67±2.251	0.5749	89.67±2.94 vs 134.17±5.037	0.5719
T0 vs T3	88.17±6.11 vs 144.50±11.221	0.0139*	86.17±4.66 vs 121.67±60.209	0.5642	89.67±3.93 vs 124.33±62.041	0.6848	89.67±2.94 vs 100.00±77.728	0.9957
T0 vs T4	88.17±6.11 vs 136.50±67.171	0.0389*	86.17±4.66 vs 120.50±94.756	0.5914	89.67±3.93 vs 141.33±112.171	0.3575	89.67±2.94 vs 108.50±118.891	0.9605
Over all group analysis T0 vs T1, T2, T3 and T4								
	Statistical test with test-value (ANVOA) F-value		p-value					
Day 7	4.369		0.0081*					
Day 14	0.7327		0.5783					
Day 21	0.8841		0.4877					
Day 28	0.4064		0.8023					

[Table/Fig-2]: Mean variations in blood glucose (mg/dL) and statistical analysis after the dose of Streptozotocin (STZ).
*p-value <0.05 was statistically significant

Groups	Days							
	7th day		14th day	p-value	21st day		28th day	
	Mean±SD	p-value	Mean±SD		Mean±SD	p-value	Mean±SD	p-value
C-peptide								
T0 vs T1	0.73±0.10 vs 0.5567±0.05125	0.0005*	0.72±0.09 vs 0.5700±0.03225	<0.0001*	0.72±0.09 vs 0.5433±0.05203	<0.0001*	0.74±0.07 vs 0.5500±0.05550	<0.0001*
T0 vs T2	0.73±0.10 vs 0.4800±0.03795	<0.0001*	0.72±0.09 vs 0.4350±0.03017	<0.0001*	0.72±0.09 vs 0.4033±0.02503	<0.0001*	0.74±0.07 vs 0.3900±0.00894	<0.0001*
T0 vs T3	0.73±0.10 vs 0.2600±0.05550	<0.0001*	0.72±0.09 vs 0.2067±0.10912	<0.0001*	0.72±0.09 vs 0.2017±0.11017	<0.0001*	0.74±0.07 vs 0.1533±0.12388	<0.0001*
T0 vs T4	0.73±0.10 vs 0.1583±0.08085	<0.0001*	0.72±0.09 vs 0.1167±0.09480	<0.0001*	0.72±0.09 vs 0.0917±0.07885	<0.0001*	0.74±0.07 vs 0.0533±0.05922	<0.0001*
Over all group analysis T0 vs T1, T2, T3 and T4								
	Statistical test with test-value (ANVOA) F-value		p-value					
Day 7	67.54		<0.0001*					
Day 14	59.62		<0.0001*					
Day 21	63.06		<0.0001*					
Day 28	86.31		<0.0001*					

[Table/Fig-3]: Mean variations in C-peptide levels (ng/mL) and statistical analysis after the dose of Streptozotocin (STZ).
*p<0.0001 was statistically highly significant

DISCUSSION

The present study showed that, there was a significant difference (decrease) in the body weight between the control group T0 versus test group T3 and T4 on 28th day after injection of STZ. Studies have shown an association between increased glucose levels and decreased body weight of diabetic animals [25-28]. Streptozotocin was used in a dose of 45 mg/kg body weight while Mozaffari et al. (1997) [25] used 90 mg/kg body weight intraperitoneally in rats, Kang N et al., used 70 mg/kg body weight intravenously in rats and Oscika TM et al., used STZ in a dose of 50 mg/kg body weight intravenously in rats for producing hyperglycaemia [25-27]. The selection of lower dose was adopted as present strain of rats could not tolerate and survive with the dose used by previous investigators. The observations and results of the present study demonstrated that STZ was effective in producing persistent hyperglycaemia in experimental animals with a dose of 55 mg/kg body weight which co related with the finding of Greg H and Terri J in 2007 [28]. The animals treated with STZ in groups T3 and T4 appeared ill-looking with loss of their body weights because of injurious effects of STZ which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions. Present observations are in agreement with the findings of Piyachaturawat P et al., Habibuddin M et al., concluded that the animals treated with STZ appeared very week with loss of their body weights because of injurious effects of STZ which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions [29,30].

The present study explained the variations of blood glucose levels in with the doses of STZ at, a varied time intervals (7th, 14th, 21st and 28th day). The route of drug administration, right dose and proper technique are key points to produce a diabetic animal model as stated by Jain DK and Arya RK who compared the different doses and routes of administration for alloxan to achieve a perfect diabetic animal model [31]. Authors used the STZ diluted in buffer solutions with pH range 4.0-5 as per many studies, administered it intraperitoneally and glycaemic levels were checked after 7th day [32,33]. It was observed in the present study that animals who received an intraperitoneal injection of STZ at 55 mg/kg dose developed a sustained hyperglycaemic profile as reported by Masashi K and Jerrold M [34]. Similar to the present study, Greg H and Terri J have also highlighted that the same dose of STZ i.e., 55 mg/kg produced hyperglycaemia without causing any harm to animals [28]. We found mortality rates in two groups T3 and T4 who received STZ 65 mg/kg and 75mg/kg, respectively. Contrary to the present results few studies have documented that single intraperitoneal injection of STZ at a dose of 100 mg/kg will produce immediate hyperglycaemia in animals without harming them [35,36]. In our study, we found high mortality rate in one group (T4) who received STZ 75 mg/kg. Zhang M et al., concluded their study by stating that 45 mg/kg dose of STZ will give a persistent diabetic profile [37], Whereas in the present study, author observed that few rats from group T1 which were intervened by 45 mg/kg dose of STZ become euglycaemic by 7th day of intervention. This can be explained as the young animals have an increased threshold of sensitivity to the potentially toxic effects of STZ. In addition, it is likely that the aggressive fluid administration after STZ injection contributed to the low morbidity and absent mortality. The present study revealed that intraperitoneal injection of STZ induced changes including hyperglycaemia and deceased C-peptide levels with an obvious evidence of diabetogenesis The possible mechanisms for β-cells destruction by STZ was reported to induce generation of some types of oxygen free radicals and alteration of endogenous scavengers of these reactive species, fragmentation of DNA and the subsequent increase in the activity of Polymeric Adenosine Diphosphate Ribose (poly-ADP) ribose synthase (an enzyme known to deplete nicotinamide adenine dinucleotide in β-cells), inhibition of Adenosine Triphosphate (ATP) synthesis and islet mitochondrial respiratory enzymes [38]. Authors found 55 mg/kg weight of STZ was ideal for inducing diabetes in albino wistar rats suitable to the experimental surroundings.

The present study explains the variations in C-peptide levels with the doses of STZ at a varied time interval (7th, 14th, 21st and 28th day). The C-peptide is a product of pro-insulin cleavage; numerous studies have demonstrated that C-peptide, although not influencing blood glucose control, may play a role in preventing and potentially reversing some of the chronic complications of diabetes [38]. Increasing evidence suggests that declining ß-cell function in diabetes and the lack of C-peptide secretion might play a putative role in the development of microvascular abnormalities, which go beyond the effects of declining insulin secretion or increased blood glucose levels in diabetes [39]. In this study, authors found that C-peptide level was reduced significantly in STZ diabetic compared with normal rats group. These findings coincide with the results of Frost T et al., and Cong L and Chen J [40,41]. The STZ diabetic rats showed a highly significant decrease in serum C-peptide level as compared to normal rats. The diabetogenic agent STZ selectively destructs β-cells of the islets of Langerhans in the pancreas result in inhibition of insulin synthesis. This can be explained as C-Peptide is produced in β-cells in the pancreas, and secreted into the blood stream. So, when there is destruction of β-cells by STZ, the secretion of C-peptide decreases.

Limitation(s)

This study was conducted for a short duration. The follow-up after giving STZ was done for 28 days only. After administration of streptozotocin authors observed the hyperglycaemic profile of animals for 28 days. Future studies with longer duration of follow-up of more than 28 days was recommended to find out the exact time of insulin reversal after administration of STZ at 55 mg/kg dose. Furthermore, histopathologic examinations should be performed immediately after STZ administration and it should be repeated after the insulin reversal to analyse the recovery of β islets from STZ toxicity.

CONCLUSION(S)

This study would help in deciding the accurate dose of STZ for induction of diabetes in wistar albino rats in our scenario. Administration of 55 mg/kg intraperitoneal dose of STZ created diabetic animal model with persistent hyperglycaemia. Dose of 75 mg/kg was found to be lethal to animals indicated by high mortality rate. STZ at 45 mg/kg dose was insufficient to create persistent diabetic profile in animal model. It may be concluded that the STZ through its direct alkylating action can cause selective destruction of the β-cells producing hyperglycaemia at a dose of 55 mg/kg body weight. It may also be stated that STZ by producing diabetes (hyperglycaemia) and decrease in C-peptide levels causes reduction in the body weight of diabetic animals. The present study has been designed to observe the effects of STZ-induced diabetes and its association between the reduction in the weights of animal.

Acknowledgement

Authors were thankful to Department of Science and Technology, Government of India for providing funding under Women Scientist Scheme No. SR/WOS-A/LS-409/2016(G), Manipal Academy of Higher Education, Palamur Biosciences Pvt. Ltd. and S.V.S. Medical College for support. Authors also thank Mr. Giri, Mr. S.Siva Shankar Prasad (Scientist, Palamur Bioscience Pvt. Ltd.), Mr. V.D. Giridhar Rao (Deputy Quality Manager, Palamur Bio Science Pvt. Ltd.) for the timely support and guidance provided in the quality control of test item analysis. We appreciate the efforts of Mr. S. Beesaiah Goud (Lab technician, S.V.S. Medical College) and Mr. Mehamood (Lab technician, S.V.S. Medical College) for the support during analysis. We appreciate the efforts and help of Mr. Suresh (Assistant In-charge Animal House, Palamur Bioscience Pvt. Ltd.) and Mr. Pradeep ((Lab

Assistant, Animal house, Palamur Bioscience Pvt. Ltd.) for the support regarding intervention and husbandry of Animals.

REFERENCES

[1] Lee SI, Kim JS, Oh SH, Park KY, Lee HG, Kim SD. Antihyperglycemic effect of Fomitopsis pinicola extracts in streptozotocin-induced diabetic rats. J Med Food. 2008;11(3):518-24.
[2] Knentz AJ, Nattras M. Diabetic ketoacidosis, nonketotic hyperosmolar coma and lactic acidosis; Handbook of diabetes, eds. By Pickup JC, Williams G. Blackwell Science.1991;(2):479-94.
[3] Kumar PJ, Clark M. Textbook of Clinical Medicine. Saunders, London. 2002; 1099-121.
[4] Singh VP, Bali A, Singh N. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 2014;18:01-14.
[5] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87(1):04-14.
[6] Etuk EU. Animals models for studying diabetes mellitus. J Agric Biol JN Am. 2010;1(2):130-34.
[7] Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward KW. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: Route of administration, pitfalls, and insulin treatment. Comp Med. 2004;54(3):252-57.
[8] Thatte U. Still in search of an herbal medicine…. Indian J Pharmacol. 2009;41(1):1.
[9] Srinivasan K, Ramarao P. Animal model in type 2 diabetes research: An overview. Indian J Med Res. 2007;125(3):451.
[10] Dolan ME. Inhibition of DNA repair as a means of increasing the antitumor activity of DNA active agents. Adv Drug Del Rev. 1997;26:105-18.
[11] Reusser F. Mode of action of Streptozotocin. J Bacteriol. 1971;105:580-88.
[12] Junod A, Lambert AE, Orci L, Pictet R, Gonet AE, Renold AE. Studies of the diabetogenic action of streptozotocin. Proc Soc Exp Biol Med. 1967;126:201-05.
[13] Holemans K, Aerts L, Van Assche FA. Fetal growth restriction and consequences for the offspring in animal models. J Soc Gynecol Investig. 2003;10(7):392-99.
[14] Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50(6):537-46.
[15] Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26.
[16] Ito M, Kondo Y, Nakatani A, Naruse A. New model of progressive non-insulin-dependent diabetes mellitus in mice induced by streptozotocin. Biol Pharm Bull. 1999;22(9):988-89.
[17] Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi SH, Farhangi A, Verdi AA, et al. Induction of diabetes by streptozotocin in rats. Indian J Clin Biochem. 2007;22(2):60-64.
[18] Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, et al. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Lab Anim. 2011;45(3):131-40.
[19] Steiner DF, Cunningham D, Spigelman L, Aten B. Insulin biosynthesis: Evidence for a precursor. Science. 1967;157:697-700.
[20] Yosten GLC, Maric-Bilkan C, Luppi P, Wahren J. Physiological effects and therapeutic potential of proinsulin C-peptide. Am J Physiol Endocrinol Metab. 2014;307:E955-68.
[21] Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 2013;30:803-17.
[22] Kulkarni CM, Patil S. Urinary C-peptide and urine C-peptide/creatinine ratio (UCPCR) are possible predictors of endogenous insulin secretion in T2DM subjects-A randomized study. Int J Pharma Bio Sci. 2016;7:443-46.
[23] Trinder P. Enzymatic determination of glucose in blood serum. Annals of Clinical Biochemistry. 1969;6(24).
[24] Gosling JP. A decade of development in immunoassay methodology. Clin Chem. 1990;36:1408.
[25] Mozaffari MS, Warren BK, Russell CM, Schaffer SW. Renal function in the non-insulin dependent diabetic rat: Effect of unilateral nephrectomy. J Pharmacol Toxicol Methods. 1997;37(4):197-203.
[26] Kang N, Alexander G, Park JK, Maasch C, Buchwalow I, Luft LC, et al. Differential expression of protein kinase C isoforms in streptozotocin-induced diabetic rats. Kidney Int. 1999;56(5):1737-50.
[27] Oscika TM, Yu Y, Panagiotopoulos S, Clavant SP, Kirizis Z, Pike, et al. Prevention ofalbuminuria by aminoguanidine or ramipril in streptozotocin-induced diabetic rats is associated with the normalization of glomerular protein kinase C. Diabetes. 2000;49(1):87-93.
[28] Greg H, Terri J. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology. 2007;12:261-66.
[29] Piyachaturawat P, Poprasit J, Glinsukon T, Warichanon C. Gastric mucosal lesions in Streptozotocin-diabetic rats. Cell Biol Intern Rep. 1988;12(1):53-63.
[30] Habibuddin M, Daghriri HA, Humaira T, Al-Qahtani MS, Hefzi AA. Antidiabetic effect of alcoholic extract of Caralluma sinaica L. on streptozotocin-induced diabetic rabbits. J Ethnopharmacol. 2008;117(2):215-20.
[31] Jain DK, Arya RK. Anomalies in alloxan-induced diabetic model: It is better to standardize it first. Indian J Pharmacol. 2011;43(1):91.
[32] Jiao Y, Wang X, Jiang X, Kong F, Wang S, Yan C. Antidiabetic effects of Morus alba fruit polysaccharides on high-fat diet-and streptozotocin-induced type 2 diabetes in rats. J Ethnopharmacol. 2017;199:119-27.
[33] Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2015;70(1):05-47.
[34] Masashi K, Jerrold M. Effects of Streptozotocin-induced diabetes on insulin binding, glucose transport, and intracellular glucose metabolism in isolated rat adipocytes. Diabetes. 1979;28(2):87-95. https://doi.org/10.2337/diab.28.2.87.
[35] Tzeng TF, Liou SS, Chang CJ, Liu IM. The ethanol extract of Zingiber zerumbet attenuates streptozotocin-induced diabetic nephropathy in rats. Evid Based Complement Alternat Med. 2013.
[36] Riya MP, Antu KA, Pal S, Srivastava AK, Sharma S, Raghu KG. Nutraceutical potential of Aerva lanata (L.) Juss. ex Schult ameliorates secondary complications in streptozotocin-induced diabetic rats. Food Funct. 2014;5(9):2086-95.
[37] Zhang M, Xiao Yan L, Jing L, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Experimental Diabetes Research. 2008;22005:704045. 9 pages. Doi: 10.1155/2008/704045.
[38] Ohkuwa T, Sato Y, Naoi M. Hydroxyl radical formation in diabetogenic rats induced by streptozotocin. Life Sci. 1995;56:1789-95.
[39] Lee SK, Lee JO, Kim JH, Jung JH, You GY, Park SH, et al. C-peptide stimulates nitrites generation via the calcium-JAK2/STAT1 pathway in murine macrophage Raw264.7 cells. Life Sci. 2010;86(23-24):863-68.
[40] Forst T, Kunt T, Wilhelm B, Weber MM, Pfützner A. Role of C-Peptide in the regulation of microvascular blood flow. Exp Diabetes Res. 2008;2008:176245.
[41] Cong L, Chen J. Effects of exercise on leptin in streptozotocin–induced diabetic rats. Wei Sheng Yan Jiu. 2001;30(3):158-59.

PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Department of Biochemistry, Manipal Academy of Higher Education, Manipal, Karnataka, India.
2. Chief Executive Officer, Department of Toxicology, Palamur Bioscience Pvt. Ltd., Mahabub Nagar, Telangana, India.
3. Director, Department of Toxicology, Palamur Bioscience Pvt. Ltd., Mahabub Nagar, Telangana, India.
4. Professor and Head, Department of Biochemistry, S.V.S. Medical College, Mahabub Nagar, Telangana, India.
5. Statistician Cum Assistant Professor, Department of Community Medicine, Palamur Bioscience Pvt. Ltd., Mahabub Nagar, Telangana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Suhasini Padugupati,
Research Scholar, Palamur Bioscience Pvt. Ltd., S.V.S. Medical College Campus,
Y Enugonda, Mahabub Nagar, Telangana, India.
E-mail: drsuhasinimd@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Sep 16, 2020
- Manual Googling: Jun 02, 2021
- iThenticate Software: Sep 30, 2021 (24%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: Funded by Government of India, KIRAN DIVISION. No. (SR/WOS-A/ LS-409 /2016(G)).
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 14, 2020**
Date of Peer Review: **Jan 04, 2021**
Date of Acceptance: **Jun 08, 2021**
Date of Publishing: **Oct 01, 2021**