Journal of Phytonanotechnology and Pharmaceutical Sciences 4(2): 1-8, 2024

Online ISSN:2583-0376

http://jpps.ukaazpublications.com

DOI: http://dx.doi.org/10.54085/jpps.2024.4.2.1

Journal of Phytonanotechnology and Pharmaceutical Sciences

Review Article : Open Access

Neonatal streptozotocin induced diabetic rat models: A superior approach to mimicking human type 2 diabetes

Suwashni Priya*, Mohd. Ibrahim Khan, Saif Mohammed Saleh Ansari***, Mohammad Taha Kazmi**** and Danish Ahmed****

**Department of Pharmaceutical Biotechnology, Delhi Pharmaceutical Sciences and Research University, New Delhi-110062, India*

*** Department of Pharmaceutical Sciences, Faculty of Health Science, SIHAS, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj-211007, Uttar Pradesh, India*

**** Department of Biochemistry and Biochemical Engineering, Sam Higginbottom University of Agriculture Technology and Sciences (SHUATS), Prayagraj-211007, Uttar Pradesh, India*

***** Department of Pharmaceutical Chemistry, School of Pharmaceutical Education and Research (SPER), Jamia Hamdard, New Delhi-110062, India*

Article Info

Article history

Received 20 April 2024

Revised 25 May 2024

Accepted 26 May 2024

Published Online 30 June 2024

Keywords

Diabetes

STZ

Insulin resistance

Neonatal

Glycemia

Diabetic rat model

Abstract

Diabetes mellitus, the world's fastest-growing disease, is increasingly pervasive across individuals, families, and nations. The primary cause of all types of diabetes lies in malfunction or destruction of pancreatic beta cells. Various factors including genetic abnormalities, insulin resistance, autoimmune responses, inflammation, and environmental influences can contribute to the loss of beta cell function or their complete destruction. Though, there are several animal models for type 2, diabetes mellitus available, including genetically modified and chemically generated animals, none of them accurately replicates the human condition. The objective of this review is to evaluate the neonatal streptozotocin-induced rat (n-STZ) model of type 2 diabetes mellitus, with a focus on its possible benefits over alternative models. When streptozotocin dosage and time are taken into account, the n-STZ model accurately depicts a number of features of type 2 diabetes mellitus, such as reduced glucose tolerance and fluctuating hyperglycemia. In n-STZ rats, β-cell activity closely resembles patterns of insulin production seen in people with type 2 diabetes. As a result, it is acknowledged that the n-STZ model is a suitable animal model to study type 2 diabetes.

1. Introduction

Diabetes mellitus, a group of protracted metabolic ailments, is characterized by elevated plasma glucose levels, known as hyperglycemia. Its pathophysiology involves impaired insulin secretion, utilization, or both, leading to abnormalities in the metabolism of carbohydrates, fats, and proteins. Complications of diabetes include retinopathy, nephropathy, and neuropathy, which can manifest if the condition is untreated (Roglic, 2016). Chronic diabetes increases the risk of peripheral artery disease, cerebrovascular disease, heart disease, obesity, cataracts, erectile dysfunction, and non-alcoholic fatty liver disease. Moreover, individuals with diabetes have an elevated susceptibility to certain infectious diseases such as tuberculosis. Diabetes is often identified by a range of specific symptoms such as excessive thirst, dry mouth, frequent urination (polyuria), blurred vision, weight loss, fatigue, slow wound healing, and sensations of tingling and numbness in the extremities, as well as recurrent fungal infections in the genital area (Alberiti *et al.,* 2007). The primary cause of all types of diabetes lies in the malfunction or destruction of pancreatic beta cells. Various factors including genetic abnormalities, insulin resistance, autoimmune responses, inflammation, and environmental influences can contribute to the loss of beta cell function or their complete destruction (Alberiti *et al.,* 2004). It is crucial to differentiate between malfunctioning beta cells and reduced beta cell mass, as this understanding holds significant implications for therapeutic approaches aimed at preserving or improving glucose tolerance (Kahn, 2014).

Insulin, generated by pancreatic beta cells located in the islets of langerhans, plays a crucial role in controlling blood sugar levels. It aids in transporting glucose from the blood into cells, where it is used for energy generation. Carbohydrates are broken down into glucose by the body, which insulin then transports into organs *via* the bloodstream. Diabetes mellitus is classified into two main types: type 1 diabetes (T1DM), also known as insulin-dependent diabetes mellitus (IDDM), and type 2 diabetes (T2DM), also known as non-insulin dependent diabetes mellitus (NIDDM) (Bilous *et al.,* 2021). T2DM poses a significant global health threat, ranking as the third leading cause of death worldwide. The prevalence of T2DM is projected to rise from 6% (246 million people) in 2007 to 7.3% (380 million people) by 2025, marking it as a growing epidemic. Beyond T1DM and T2DM, other clinically identifiable subtypes include gestational diabetes, monogenic forms (*e.g.*, maturity-onset diabetes of the young (MODY) or neonatal diabetes), and possibly a late onset autoimmune variant known as latent autoimmune diabetes in adults (LADA) (Cole and Florez, 2020)

Corresponding author: Dr. Danish Ahmed

Associate Professor, Department of Pharmaceutical Sciences, Faculty of Health Science, SIHAS, Sam Higginbottom University of Agriculture, Technology & Sciences (SHUATS), Prayagraj-211007, Uttar Pradesh, India

E-mail: danish.ahmed@shiats.edu.in

Tel.: +91-9580001578

1.1 Prevalence of diabetes

Diabetes mellitus, the world's fastest-growing disease, is increasingly pervasive across individuals, families, and nations. According to the IDF diabetes Atlas 2021, approximately 10.5% of adults aged 20-79 have diabetes, with more than half unaware of their condition. Type 2 diabetes, which constitutes around 90% of all diabetes cases globally, is primarily driven by environmental, genetic, socio-economic, and demographic factors. Factors contributing significantly to the increasing prevalence of type 2 diabetes include urbanization, an ageing population, reduced levels of physical activity, and rising rates of overweight and obesity (IDF, 2021). Projections by the IDF suggest that by 2045, approximately 783 million adults, or one in eight, will have diabetes, a 46% increase from current levels. At present, approximately 537 million adults aged 20-79 have diabetes, with projections indicating this number will increase to 643 million by 2030 and 783 million by 2045. One-third of adults diagnosed with diabetes live in low to middle-income countries (IDF, 2021).

Table 1: Various models and methods of diabetes induction with concise mechanisms

Model/Animal	Induction mode	Occurrence	Mechanism	References
Alloxan induced diabetes	Chemically induced diabetes	Type 1 diabetes	Targeted suppression of insulin release triggered specifically by glucose	Rohilla and Ali, 2012
Streptozotocin induced diabetes (Multiple low doses)			Injures pancreatic β cells, leading to reduced insulin production and elevated blood glucose levels.	Gvazava *et al.*, 2018
Streptozotocin induced diabetes (Low-dose of STZ with high fat diet)		Type 2 diabetes		Reed *et al.*, 2000
Streptozotocin induced diabetes (STZ-Nicotinamide)				Ahmed *et al.*, 2017
Streptozotocin induced neonatal diabetes (n0-STZ)				Portha *et al.*, 1974
Streptozotocin induced neonatal diabetes (n2-STZ)				Weir *et al.*, 1981
Streptozotocin induced neonatal diabetes (n5-STZ)				Wang *et al.*, 1996
Pancreatectomy model	Surgical induced diabetes		The pancreatic beta cell mass decreases when a significant portion of the pancreas is surgically removed.	Chen *et al.*, 2016
Coxsackie B virus induced mode	Virus induced diabetes	Type 1 diabetes	Leads to the onset of insulin-dependent diabetes mellitus due to reactivation of auto-reactive T cells.	Horwitz *et al.*,1998
EMC virus induced mode			Develops diabetes mellitus through targeted destruction of β cells.	Filippi and Herrath, 2008
Goto Kakizaki rat	Genetically modified induced diabetes models	Type 2 diabetes	Polygenic model predisposing individuals to early-onset type 2 diabetes mellitus.	Wang *et al.*, 2013
BB rat		Type 1 diabetes	Spontaneously exhibit hyperglycemia and ketoacidosis, hallmarking the clinical onset of type 1 diabetes.	King, 2012
Zucker diabetic fatty rat		Type 2 diabetes	Developed through a missense mutation in the leptin receptor gene, this model manifests obesity without diabetes and is employed in the study of type 2 diabetes.	Wang *et al.*, 2014
AKITA		Type 1 diabetes	Genetically induced monogenic model that develops insulin-dependent diabetes mellitus.	King, 2012

1.2 Diabetes in India

India, a nation undergoing rapid urbanization and socio-economic development, is significantly impacted by the global diabetes epidemic. Research conducted across India indicates a rising prevalence of diabetes as urbanization influences lifestyle factors in both urban and rural populations. Prediabetes also poses a substantial health risk. It is estimated that over 77 million Indians aged 18 and

above have type 2 diabetes, with an additional 25 million in the prediabetic stage, predisposing them to future diabetes onset (Pradeepa and Mohan, 2021). Alarmingly, more than half of these individuals are unaware of their condition, potentially leading to severe health complications if left untreated. Adults with diabetes face a significantly higher risk of heart attacks and strokes, underscoring the urgent need for early detection and intervention (IDF, 2021).

Research conducted in 2021 by the ICMR-NMMS group reveals significant prevalence rates of impaired fasting blood glucose (IFG) and diabetes mellitus (DM) in India, at 9.3% and 24.5%, respectively. Among individuals diagnosed with DM, only 15.7% have their condition effectively managed, while 36.1% are receiving medication, and 45.8% are aware of their diabetes status (Mathur *et al.,* 2022). The majority of diabetes patients seek treatment from allopathic practitioners, reflecting the predominant healthcare-seeking behavior among adults. Elderly individuals show heightened awareness of diabetes and are at increased risk of developing the disease. Those with elevated blood pressure and cholesterol exhibit better levels of awareness, treatment, and management (Mathur*et al.,*2022). Looking forward, the World Health Organization (WHO) predicts a significant rise in urban populations in developing countries, with India expected to have approximately 46% of its population residing in urban areas by 2030. This demographic shift underscores the importance of addressing urbanization's impact on lifestyle-related diseases like diabetes (IDF, 2021).

2. Handling and animal models for diabetes

Given the widespread occurrence of diabetes mellitus worldwide, the advancement of effective therapeutic approaches is critically important. The process of drug development involves several stages, with the pre-clinical phase playing a crucial role. During this phase, animal models are instrumental in closely mimicking the clinical manifestations of diseases such as diabetes in humans. These models are essential for unravelling the complex mechanisms underlying diabetes mellitus and for evaluating potential therapies before they advance to clinical trials (Martín-Carro *et al.,* 2023). To facilitate the discovery of novel treatments for diabetes, the initial step often involves inducing diabetes in animal models that replicate human diabetes. There are various methods and models utilized in laboratory settings to induce diabetes (Table 1), several of which are outlined below:

2.1 Chemically induced diabetes model

Animal models are essential in diabetes research, particularly through chemically induced diabetes in rats using diabetogenic agents. Two commonly used chemicals for this purpose are alloxan and streptozotocin, both administered through parenteral routes. The dosage of these drugs varies based on the specific animal species and the method of administration (Lanzen and Panten, 1988; Szkudelski, 2001). These agents are crucial for studying the pathophysiology of diabetes and testing potential therapeutic interventions in controlled experimental settings.

2.2 Alloxan-induced diabetes model

Alloxan, chemically known as 5,5-dihydroxyl pyrimi-dine-2,4,6-trione, is an organic compound analog of cyclic urea. Alloxan was the pioneering agent introduced for inducing diabetes in laboratory animals (Lanzen and Panten, 1988). Its effects on inducing diabetes follow a triphasic time course response. Initially, alloxan causes a rapid increase in blood glucose levels, succeeded by a decline likely due to depletion of insulin by beta cells. Subsequently, there is a prolonged elevation in blood glucose levels (Rohilla and Ali, 2012).

2.2.1 Diabetes can be triggered by alloxan through two primary mechanisms

i. Alloxan selectively inhibits the beta-cell pancreatic glucose sensor, specifically the wide glucokinase curb, crucial for triggering insulin secretion when glucose levels rise (Katoh*et al.,*2002).

ii. Alloxan triggers the production of reactive oxygen species (ROS), initiating a redox cycle that generates superoxide radicals. This cycle involves reduction to dialuric acid and subsequent re-oxidation to alloxan, resulting in the production of superoxide radicals. Superoxide dismutase neutralizes these radicals, converting them into hydrogen peroxide, and may also lead to the formation of hydroxyl radicals through secondary reactions. (Dolan, 1997). Importantly, alloxan's hepatotoxicity is minimal due to the liver's robust biotransformation systems and superior ROS defence mechanisms compared to beta cells.

iii. Additionally, alloxan disrupts the balance of intracellular calcium, causing elevated calcium levels that result in cellular damage over time. It also encourages the oxidation of sulfhydryl groups, particularly targeting molecules like glutathione (GSH) and proteins (King, 2012). Alloxan is administered at dosages ranging from 40 to 200 mg/kg for rodents and 50 to 200 mg/kg for mice, with variations based on strain and method of administration (*e.g.*, intraperitoneal or subcutaneous). Higher doses are required for non-intravenous routes of administration.

2.3 Streptozotocin-induced diabetes model

Streptozotocin, also known by its chemical name N-(methyl nitrosocarbamoyl)-α-d-glucosamine, is an antibacterial compound naturally produced by Streptomyces achromogenes (Wu and Yan, 2015). It is recognized as the best diabetogenic agent due to its cytotoxic effects (Rakieten*et al.,*1969) on beta cells in the pancreas, similar to alloxan. This compound is widely employed to induce diabetes in experimental animal models (Ventura-Sobrevilla *et al.,* 2011).

2.3.1 Proposed mechanism of STZ toxicity

While the precise mechanism of STZ's toxic effects remains debated, one suggested action is its interaction with nuclear DNA. STZ undergoes decomposition, forming highly reactive carbonium ions that can alkylate DNA bases. Additionally, STZ may damage β-cell membranes and induce DNA strand breaks, triggering the activation of poly (ADP-ribose) synthetase and depletion of NAD. These processes ultimately lead to cell death (Arulmozhi *et al.,* 2004).

2.3.2 Streptozotocin exerts its diabetogenic effects through two distinct mechanisms

i. At higher doses, Streptozotocin selectively targets pancreatic beta cells, a characteristic typical of cytotoxic nitrosourea compounds. Despite its hydrophilic nature, unlike most nitrosourea compounds that are lipophilic, Streptozotocin is transported into beta cells *via* the glucose transporter protein GLUT-2, owing to its structural similarity to glucose (Graham *et al.,* 2011).

ii. At lower doses administered over time, streptozotocin induces the release of the enzyme glutamic acid decarboxylase, triggering an inflammatory and immunological response. This enzyme serves as a significant autoantigen in autoimmune diabetes, leading to the destruction of beta cells and subsequent hyperglycemia associated with inflammatory infiltrates, particularly pancreatic lymphocytes (Elsener *et al.,* 2000).

Interestingly, while streptozotocin selectively targets beta cells in the pancreas, it spares alpha cells and other pancreatic cell types from damage. This specificity holds true in human studies as well, where streptozotocin does not affect any pancreatic cells besides beta cells (Furman, 2015).

In animal studies, high doses of streptozotocin (ranging from 100 to 200 mg/kg in mice or 35 to 65 mg/kg in rats) result in extensive beta cell death and a subsequent lack of insulin production (Furman, 2021). Conversely, lower doses (typically 20 to 40 mg/kg/day) administered over an unspecified period induce inflammation at varying concentrations, contributing to experimental diabetes models (Thayer *et al.,* 2010).

2.4 High-fat dietinduced-diabetes model

The rodent model fed with a high fat regime has become a pivotal tool for studying type 2 diabetes mellitus. First introduced in 1988 by Surwit and colleagues.This model has been instrumental in illustrating insulin resistance and insufficient islet compensation during intravenous glucose tolerance investigations. It has been extensively utilized to investigate the pathogenesis of type 2 diabetes, impaired glucose tolerance (IGT), and to explore novel therapeutic strategies (Surwit *et al.,* 1988).

In this model, female C57BL/6J mice are typically employed. Shortly after arrival, these mice are divided into two groups: one group receives a standard diet continuously for up to a year, while the other group is fed a high fat diet. The composition of these diets differs significantly: the standard diet consists of 12.6 kJ/g total protein, 62.8% carbohydrates, and 5.8% fat derived from lard. In contrast, the high-fat diet contains 16.4% protein, 5.8% fat from lard, and 25.6% carbohydrates, totalling 23.4 kJ/g. Throughout the study period, researchers monitor food consumption and body weight weekly (Ahren and Pacini, 2002). Blood samples are collected from the intraorbital retrobulbar plexus of anesthetized, non fasting mice at specified intervals for analysis.This experimental approach allows researchers to observe metabolic changes, insulin sensitivity, and glucose regulation dynamics in response to prolonged high fat dietary intake, mimicking aspects of human type 2 diabetes progression. It serves as a robust platform for investigating both disease mechanisms and potential therapeutic interventions (Winzell and Ahren, 2004).

2.5 Virus induced-diabetes model

Viral infections can also induce diabetes in laboratory animals. The EMC virus, reovirus types 1 and 3, and coxsackie virus B4 have been observed to cause pancreatic islet necrosis in mice (Jun and Yoon, 2003). Research has examined various strains, ages, and sexes of mice to understand how these viruses impact pancreatic β cells under diverse conditions. Research has also investigated the impact of congenital rubella infection in rabbits (Kang *et al.,* 1994). Some viruses have been found to damage β-cells and induce diabetes, while others may have protective effects against diabetes. The development of diabetes due to viruses depends not only on the virus's ability to induce the disease but also on the vulnerability of the host entity. Consequently, creating an animal model that accurately mimics virus prompted diabetes in homo sapiens is challenging. The relationship between viral infection and the development of type 1 diabetes mellitus remains uncertain and requires further investigation. Given these complexities, developing a suitable model for virus induced diabetes is likely to attract significant research attention in the future. Notably, within the past decade, only 2% of articles focused on diabetic animal models have explored virus induced models (Ellerman *et al.,* 1996).

2.6 Surgical induced-diabetes models

This model is created by involving the pancreatic ducts surgically or by partially or completely removing the pancreas. These methods are not commonly employed due to their invasive nature, but they are utilized in studies concerning pancreas transplantation (Omori *et al.,* 2016).

2.7 Genetically modified induced-diabetes models

2.7.1 ZDF rat model

The ZDF rats, also known as zucker fatty diabetic rats, are obese due to a mutation in the leptin receptor gene, which causes them to overeat (hyperphagia). They develop hyperlipidemia and hyperinsulinemia, but typically maintain normal blood glucose levels and rarely progress to mild hyperglycemia (Todd, 2016). These characteristics mirror the prediabetics state in humans, where obesity is a significant risk factor for type-2 diabetes development. Through selective breeding of ZF rats, the ZDF rats were created. Unlike ZF rats, ZDF rats exhibit severe insulin resistance and progressively develop hyperglycemia, with levels exceeding 500 mg/dl by week 10. This model spontaneously develops type 2 diabetes more frequently in male rats. Despite genetic differences between these rats and humans, they manifest similar complications in advanced disease stages, such as glomerular lesions, meningeal matrix expansion, and tubule interstitial fibrosis, among others. Consequently, this model has been valuable for studying alterations associated with advanced stages of type 2 diabetes.

2.7.2 Goto-Kakizaki rat model

The Goto-kakizaki (GK) rats are a well-known model for type 2 diabetes that differs from other models by not exhibiting obesity or hyperlipidemia. They are derived from the selective breeding of wistar rats with impaired glucose tolerance. By 12 weeks of age, GK rats develop hyperglycemia, hypoinsulinemia, and peripheral insulin resistance. Exposure of the pregnant rat's fetus to a hyperglycemic environment appears to impact the normal development of beta cells, resulting in reduced pancreatic islet numbers at birth. Interestingly, exercise has been shown to mitigate glycemic increases in these rats. This model shares certain environmental factors with human type 2 diabetes, such as intrauterine hyperglycemia and the influence of physical activity, making it attractive for research on type 2 diabetes prevention and treatment. GK rats also develop abnormalities in the retina, kidneys, and peripheral nerves, which makes them valuable for studying disease-related complications. However, limitations include a low rate of successful pregnancies and fewer rats per litter, which can restrict their use in research studies (Goto *et al.,*1976).

2.7.3 BB rat model

The Bio-Breeding diabetic-prone (BB-DP) rats were discovered in the 1970s at Bio-Breeding Laboratories in Canada, originating from a spontaneous mutation in a wistar rat colony affecting the major histocompatibility complex (MHC). Both male and female rats develop type 1 diabetesat similar rates, typically between 50 and 90 days after birth, characterized initially by severe pancreatic insulitis leading to hypoinsulinemia. The first sign of disease is glycosuria at 8-16 weeks, with 90% of the rats progressing to overt type 1 diabetes marked by hyperglycemia, weight loss, polyuria, polydipsia, and severe ketoacidosis necessitating exogenous insulin for survival (Hartoft-Nielsen *et al.,*2009). In contrast, Bio-Breeding diabetes-resistant rats do not develop diabetes mellitus and serve as controls. Despite similarities to type 1 diabetesin humans, a notable limitation of the BB-DP model is the concurrent T-cell reduction, a condition not seen in humans or other animal models, which has raised questions about its relevance. Furthermore, certain promising antidiabetic therapies, like anti-CD3 therapy, have shown T-lymphocyte depletion as a side effect, precluding their study using this model (Prins *et al.,*1991). Despite these drawbacks, BB-DP rats have been extensively utilized to investigate diabetes mellitus pathophysiology and islet transplantation.

Various animal models of type 1 and type 2 diabetes serve distinct purposes, including pharmacological testing, genetic studies, and elucidating disease mechanisms. The choice of model depends on the specific objectives of the research. For instance, when testing pharmacological agents, the mechanism of action of the drug under investigation guides the selection of an appropriate animal model.

2.7.4 Neonatal induced diabetic models

Among these models, the neonatal STZ (n-STZ) induced rat model is frequently utilized as a type 2 diabetes like model (Portha *et al.,*1974). This model mimics human diabetes (Portha *et al.,*2009), demonstrating characteristics such as initial destruction and subsequent regeneration of pancreatic beta cells leading to glucose intolerance (Bonner-Weir *et al.,*1981). Further studies have corroborated these findings, showing that rats treated with n-STZ in infancy develop typical features of type 2 diabetes in adulthood (Tourrel *et al.,* 2001). Researchers primarily use this model to evaluate potential hypoglycemic or antidiabetic agents, as well as for studies related to hypolipidemia and oxidative stress.

The n-STZ model is extensively utilized in research to induce diabetes mellitus in animals, predominantly rodents such as rats or mice. In this model, STZ, a substance toxic to insulin producing beta cells in the pancreas, is administered shortly after birth to newborn animals (Junod *et al.,*1969). This administration leads to the manifestation of symptoms resembling diabetes, including hyperglycemia (elevated blood sugar levels), insulin deficiency, and occasionally impaired glucose tolerance (Figure 1). The neonatal STZ induced rat model of type 2 diabetes mellitus involves inducing diabetes in wistar rats shortly after birth (n0 = birth) (Portha *et al.,*1979). This is achieved by administering 100 mg/kg of STZ either intravenously (*via* the saphenous vein) or intraperitoneally. Additionally, variants of the n-STZ rat model are created by administering STZ on different days post birth, such as the 2nd day (n2-STZ model) or the 5th day (n5-STZ model) (Blondel *et al.,*1989).

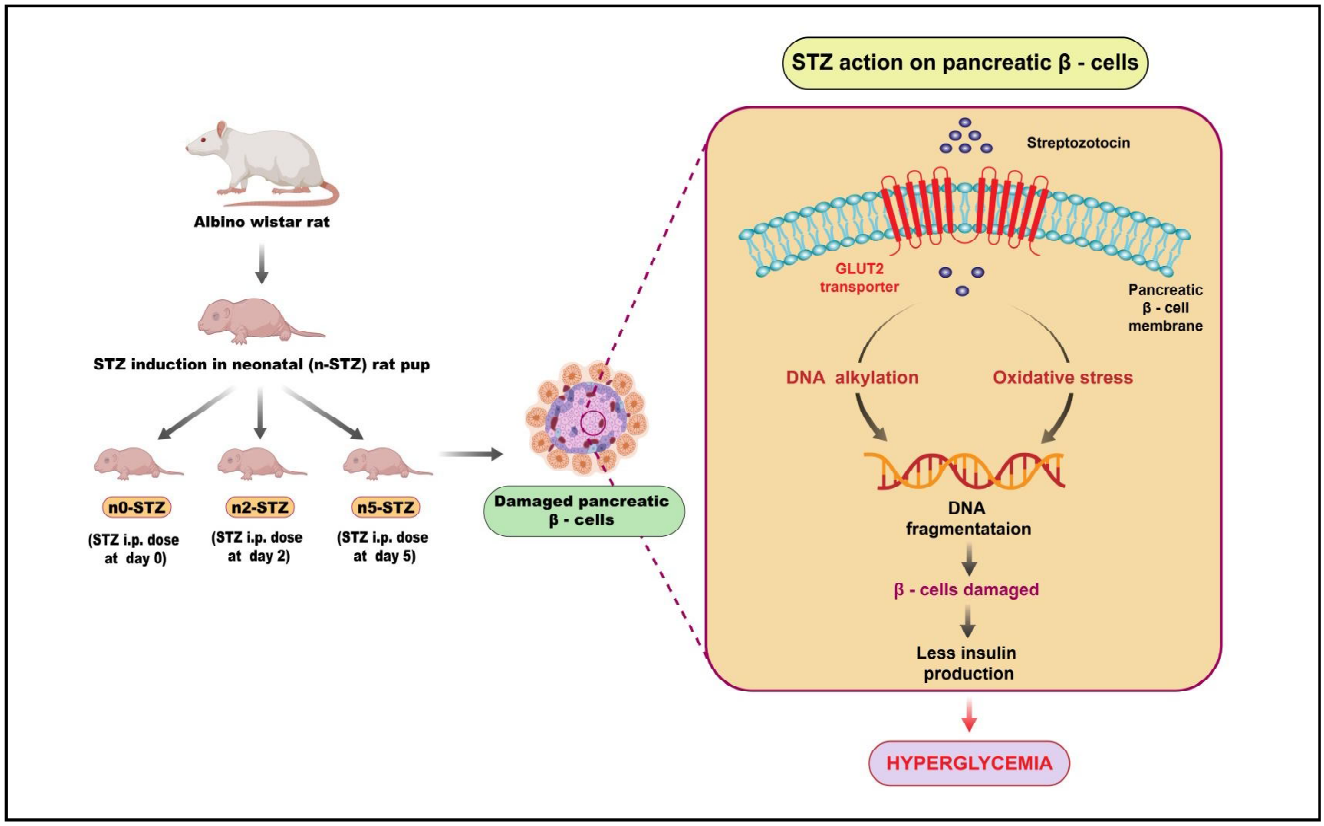

Figure 1: Schematic diagram illustrating the mechanism of streptozotocin (STZ) in neonatal pups.

In the n0-STZ model, rats treated with STZ on the day of birth develop insulin deficient acute diabetes mellitus within 3-5 days after birth. These rats exhibit high plasma glucose levels, a significant decrease of about 93% in plasma insulin levels, and elevated plasma glucagon content. However, the hyperglycemia observed in these neonates following STZ administration is transient. Research indicates that neonatal rats initially show resistance to STZ, as their plasma glucose and insulin levels eventually become comparable to control groups. By 8 weeks of age and beyond, n0-STZ rats begin to display mild hyperglycemia.

In addition to the n0-STZ model, other variants like the n2-STZ and n5-STZ models are established by injecting 80 mg/kg of STZ intraperitoneally on the 2nd and 5th days after birth, respectively. An intriguing variation involves Sprague-Dawley pups receiving 90 mg/kg of STZ on the 2nd day and 120 mg/kg on the 1.5th day after birth *via* intraperitoneal injection. By 6 weeks of age, these animals exhibit basal hyperglycemia and impaired glucose tolerance.

Comparative analysis shows that the n0-STZ and n2-STZ Wistar models are nearly identical in terms of growth patterns, basal plasma glucose and insulin levels, lack of insulin release in response to glucose *in vivo*, glucose intolerance, and depletion of pancreatic insulin stores. In the n5-STZ Wistar model, several characteristics are observed: there is sustained basal hyperglycemia, impaired glucose tolerance, elevated glycosylated hemoglobin levels, a significant reduction in pancreatic insulin stores, a 50% decrease in basal insulin levels, and a lack of plasma insulin response to glucose *in vivo*. The development and progression of hyperglycemia in the n5-STZ Wistar model show notable similarities to those seen in the n2-STZ Sprague-Dawley model.

3. Advantages of the n-STZ Model in type 2 diabetes research

The n-STZ model is preferred over other models for type 2 diabetes mellitus due to several advantages. Unlike chemically induced diabetic models that typically result in type 1 diabetes, which has a high mortality rate and requires a high dose of STZ, the n-STZ model demonstrates type 2 diabetes using a lower dose of STZ and with a reduced mortality rate through split dosing (Srinivasan *et al.,* 2005). Surgical models, which involve dissecting α and β cells, are associated with a high mortality rate, require surgical expertise, and increase the risk of digestive system impairment and inadequate response to hyperglycemia (Baig and Panchal, 2020).

In contrast, transgenic or knockout diabetes models allow researchers to study the effects of specific genes or mutations, whereas the n-STZ model enables investigation into the impact of medications on the expression of all genes without needing complex maintenance setups. The n-STZ model has been extensively studied and replicates key features of human type 2 diabetes, including chronic hyperglycemia and insulin release patterns similar to those observed in Goto-kakizaki (GK) mice, a genetically modified rat model of type 2 diabetes. Moreover, the n-STZ model is cost effective, making it accessible to researchers with limited funding (Baig and Panchal, 2020).

4. β-cell recovery in n-STZ rodents

It has been observed that following the n0-STZ infusion, signs of recovery become evident from post pregnancy day 4 onward. This recovery is characterized by the presence of various insulin positive cells scattered throughout the acinar parenchyma and within the ductal epithelium. However, in 4 month old animals, the regeneration process appeared incomplete. The efficacy of this regeneration process hinges on the timing of the STZ injection relative to the normal growth phase of the rat's islet cell mass. Two weeks after the cellular insult, the pancreas of n5-STZ rats showed negligible insulin re-accumulation, whereas n2-STZ and n0-STZ rats exhibited some restoration of their insulin stores (Weir *et al.,*1981).

Research has shown that the neonatal rat pancreas possesses a limited ability for cell regeneration, a capability that is lacking in adult rodents. In the wistar strain, this regenerative capacity declines rapidly during the first week after birth and becomes insignificant thereafter. Additionally, it is noted that cell regeneration in Sprague-Dawley neonates is less efficient compared to the wistar strain.The recovery from diabetes mellitus in the Sprague-Dawley n2-STZ model is attributed to the partial replenishment of the β-cell mass through the replication of existing β-cells, rather than through neogenesis from undifferentiated precursors (Giroix *et al.,*1983).

The n5-STZ rodents did not exhibit significant recovery of insulin in the pancreas fourteen days after β-cell damage, whereas some degree of insulin restoration was observed in the pancreas of n2-STZ and n0-STZ rodents. Research has shown that the pancreas of neonatal rats possesses some ability for cell regeneration, a capability absents in adult rodents. This regenerative potential declines rapidly in the wistar strain during the first week after birth and becomes negligible thereafter. Moreover, it is noted that cell regeneration in newborn Sprague-Dawley rats is less efficient compared to the wistar strain. In contrast to neogenesis from undifferentiated precursors, the recovery from diabetes mellitus in the Sprague-Dawley n2-STZ model is attributed to partial replenishment of β-cell mass through replication of existing cells (Hellerstrom *et al.,*1976).

5. Is the n-STZ rat a superior diabetes type 2 model?

The suitability of the n-STZ rat model as a superior type 2 diabetes model depends on various factors and the specific research objectives. STZ is commonly used to induce diabetes in animal models by damaging pancreatic β-cells, mimicking aspects of type 1 diabetes. However, when used in neonatal or adult rats, it can also induce characteristics similar to type 2 diabetes, particularly in terms of insulin resistance and β-cell dysfunction.

5.1 Here are some considerations for evaluating the n-STZ rat model as a type 2 diabetes model

i. **Relevance to type 2 diabetes mechanisms:** Type 2 diabetes involves insulin resistance and β cell dysfunction. The n-STZ model can replicate these aspects, making it suitable for studying certain aspects of type 2 diabetes pathophysiology.

ii. **Strengths in research:** The n-STZ model allows researchers to study the effects of insulin resistance, β-cell dysfunction, and potential therapeutic interventions in a controlled experimental setting.

iii. **Limitations:** While the n-STZ model has advantages, it may not fully replicate all aspects of human type 2 diabetes, such as the chronic progressive nature and the full spectrum of metabolic disturbances seen in patients.

iv. **Comparative considerations:** Researchers often choose between various animal models (*e.g.,* genetic, diet-induced, chemical-induced) based on specific research questions and the closest resemblance to human disease.

v. **Ethical and practical considerations:** Ethical considerations regarding animal use and welfare, as well as practical aspects such as cost, availability, and ease of maintenance, also influence the choice of animal models.

6. Conclusion

Experimental models of diabetes mellitus are indispensable tools in biomedical research for gaining deeper insights into the pathogenesis and refining management strategies for diabetes. However, it is lamentable that there are currently no animal models that precisely 100% mirror the complexities observed in human diabetes mellitus. It is comprehended that while none of the current animal models fully replicate type 2 diabetes mellitus in humans, the neonatal administration of streptozotocin (n-STZ) appears to more closely mimic the chronic complications and delayed onset of the disease observed in humans. As previously reported n-STZ rat models offers several advantages over other models and are considered one of the most suitable experimental animal models for studying type 2 diabetes mellitus.

Acknowledgments

Thankfulness to the authorities of Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj (Allahabad).

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Ahmed, D.; Khan, M.I.; Kaithwas, G.; Roy, S.; Gautam, S.; Singh, M.; Devi, U.; Yadav, R.; Rawat, J. and Saraf, S. (2017). Molecular docking analysis and antidiabetic activity of rifabutin against STZ-NA induced diabetes in albino wistar rats. Beni-Suef University J.Basic App. Sci., **6**(3):269-284.

Ahren, B. and Pacini, G. (2002). Insufficient islet compensation to insulin resistance vs. reduced glucose effectiveness in glucose-intolerant mice. **Am. J. Physiol. Endocrinol. Metab., 283**(4):E738-E744.

Alberti, G.;Zimmet, P.; Shaw, J.; Bloomgarden, Z.; Kaufman, F.;Silink, M. and Consensus Workshop Group. (2004). Type-2 diabetes in the young: The evolving epidemic: The international diabetes federation consensus workshop. Diabetes Care, **27**(7):1798-1811.

Alberti, K. G. M. M., Zimmet, P. and Shaw, J. (2007). International Diabetes Federation: A consensus on type 2 diabetes prevention. Diabetic Med., **24**(5):451-463.

Arulmozhi, D. K.; Veeranjaneyulu, A. and Bodhankar, S. L. (2004). Neonatal streptozotocin-induced rat model of TYPE 2 diabetes mellitus: A glance. Indian J. Pharmacol., **36**(4):217-221.

Baig, M. A. and Panchal, S. S. (2020). Streptozotocin-induced diabetes mellitus in neonatal rats: An insight into its applications to induce diabetic complications. Curr. Diabetes Rev., **16**(1):26-39.

Bilous, R., Donnelly, R. and Idris, I. (2021). Handbook of diabetes.John Wiley and Sons.

Blondel, O.; Bailbé, D. and Portha, B. (1989). Relation of insulin deficiency to impaired insulin action in NIDDM adult rats given streptozocin as neonates. Diabetes, **38**(5):610-617.

Bonner-Weir S.; Trent.DFHoney R.N. and Weir, G.C. (1981). Responses of neonatal rat islets to streptozotocin: Limited B-cell regeneration and hyperglycemia. Diabetes, **30**:64-69.

Chen, C., Cohrs; C. M., Stertmann, J.;Bozsak, R.and Speier, S. (2017). Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis. Mol. Metab., **6**(9):943-957.

Cole, J. B. and Florez, J. C. (2020). Genetics of diabetes mellitus and diabetes complications. Nature Rev. Nephrol., **16**(7):377-390.

Ellerman, K.E.; Richards, C.A.;Guberski, D.L.;Shek, W.R.; Like, A.A. (1996). Kilham rat triggers T-cell-dependent autoimmune diabetes in multiple strains of rat. Diabetes, **45**:557-562.

Elsner, M.;Guldbakke, B.;Tiedge, M.; Munday, R. and Lenzen, S. (2000). Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetologia, 43:1528-1533.

Filippi, C. M. and von Herrath, M. G. (2008). Viral trigger for type 1 diabetes: Pros and cons. Diabetes, **57**(11):2863-2871.

Furman, B. L. (2015). Streptozotocin induced diabetic models in mice and rats. Curr. Protoc. Pharmacol., **70**(1):5-47.

Furman, B. L. (2021). Streptozotocin induced diabetic models in mice and rats. Curr. Protoc., **1**(4):e78.

Giroix, M. H.; Portha, B.; Kergoat, M.; Bailbe, D. and Picon, L. (1983). Glucose insensitivity and amino-acid hypersensitivity of insulin release in rats with non-insulin-dependent diabetes: a study with the perfused pancreas. Diabetes, **32**(5):445-451.

Goto, Y.; Kakizaki, M. and Masaki, N. (1976). Production of spontaneous diabetic rats by repetition of selective breeding. The Tohoku J. Exp. Med., **119**(1):85-90.

Graham, M. L.; Janecek, J. L.; Kittredge, J. A.;Hering, B. J. and Schuurman, H. J. (2011). The streptozotocin-induced diabetic nude mouse model: Differences between animals from different sources. Comp. Med., **61**(4):356-360.

Gvazava, I. G.; Rogovaya, O. S.; Borisov, M. A.; Vorotelyak, E. A. and Vasiliev, A. V. (2018). Pathogenesis of Type 1 diabetes mellitus and rodent experimental models. Acta Naturae, **10**(1):24-33.

Hartoft-Nielsen, M. L.; Rasmussen, A. K.; Bock, T.; Feldt-Rasmussen, U.; Kaas, A. and Buschard, K. (2009). Iodine and tri-iodo-thyronine reduce the incidence of type 1 diabetes mellitus in the autoimmune-prone BB rats. Autoimmunity, **42**(2):131-138.

Hellerström, C.; Andersson, A. and Gunnarsson, R. (1976). Regeneration of islet cells. Actaendocrinologica. Supplementum, 205:145-160.

Horwitz, M. S.; Bradley, L. M.;Harbertson, J.;Krahl, T.; Lee, J. and Sarvetnick, N. (1998). Diabetes induced by Coxsackie virus: Initiation by bystander damage and not molecular mimicry. Nature Med., **4**(7):781-785.

International Diabetes Federation (2021). *IDF Diabetes Atlas* (10th ed.). Brussels, Belgium: International Diabetes Federation.

Jun, H.S. and Yoon, J.W. (2003). A new look at viruses in Type 1 diabetes. Diabetes Metab. Res. Rev., 19:8-31.

Junod, A.; Lambert, A. E.;Stauffacher, W. and Renold, A. E. (1969). Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. The J. Clin. Invest., **48**(11):2129-2139.

Kahn, S. E.; Cooper, M. E. and Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future. The Lancet, **383**(9922):1068-1083.

Kang, Y.; Chatterjee, N.K.; Nodwell, M.J. and Yoon, J.W. (1994). Complete nucleotide sequence of a strain of coxsackie B4 virus of human origin that induces diabetes in mice and its comparison with non-diabetogeniccoxsackie B4 JBV strain. J. Med.Virol., **44**:353-361.

Katoh, M., Sakurai, K. and Fujimoto, Y. (2002). Alloxan radical-induced generation of reactive oxygen species in the reaction system of alloxan with ascorbate. Yakugaku Zasshi: J. Pharm. Soc. Jpn., **122**(10):831-839.

King, A. J. (2012). The use of animal models in diabetes research. British J. Pharmacol., **166**(3):877-894.

Lenzen, S.and Panten, U. (1988). Alloxan: History and mechanism of action. Diabetologia, **31**:337-342.

Martín-Carro, B., Donate-Correa, J., Fernández-Villabrille, S., Martín-Vírgala, J., Panizo, S., Carrillo-López, N., Martínez-Arias, L., Navarro-González, J.F., Naves-Díaz, M. and Fernández-Martín, J.L., (2023). Experimental models to study diabetes mellitus and its complications: Limitations and new opportunities. Int. J. Mol. Sci., **24**:10309.

Mathur, P.; Leburu, S. and Kulothungan, V. (2022). Prevalence, awareness, treatment and control of diabetes in India from the countrywide national NCD monitoring survey. Front. Public Health, **10**:748157.

Okamoto, H. (1981). Regulation of proinsulin synthesis in pancreatic islets and a new aspect to insulin-dependent diabetes. Mol. Cellular Biochem., **37**:43-61.

Omori, K.; Kobayashi, E.; Rawson, J.; Takahashi, M. and Mullen, Y. (2016). Mechanisms of islet damage mediated by pancreas cold ischemia/ rewarming. Cryobiology, **73**(2):126-134.

Portha, B.; Lacraz, G.; Kergoat, M.; Homo-Delarche, F.;Giroix, M.H. and Bailbe D (2009). The GK rat beta cell: A prototype for the diseased human beta-cell in type 2 diabetes? Mol. Cell Endocrinol., 297:73-85.

Portha, B.;Levacher, C., Picon, L. andRosselin, G. (1974). Diabetogenic effect of streptozotocin in the rat during the perinatal period. Diabetes, **23**:889-895.

Portha, B.;Picon, L. and Rosselin, G. (1979). Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes. Diabetologia, **17**:371-377.

Pradeepa, R. and Mohan, V. (2021). Epidemiology of type 2 diabetes in India. Indian J. Ophthalmol., **69**(11):2932-2938.

Prins, J. B.; Herberg, L.; Den Bieman, M. G. C. W. and Van Zutphen, L. F. M. (1991). Genetic variation within and between lines of diabetes-prone and non-diabetes-prone BB rats; allele distribution of 8 protein markers. Laboratory Animals, **25**(3):207-211.

Rakieten, N.; Nadkarni, M. V.; Rakieten, M. L. and Gordon, B. S. (1969). Toxicologic and pharmacologic evaluation of phleomycin including special studies on its nephrotoxicity. Toxicol. Appl. Pharmacol., **14**(3):590-602.

Reed, M. J.; Meszaros, K.; Entes, L. J.; Claypool, M. D.; Pinkett, J. G. and Gadbois, T. M.(2000). A new rat model of type 2 diabetes: The fat-fed, streptozotocin-treated rat. Metab., **49**:1390-1394.

Roglic, G. (2016). WHO Global report on diabetes: A summary. Int. J. Noncommunicable Dis., 1(1):3-8.

Rohilla, A. and Ali, S. (2012). Alloxan induced diabetes: Mechanisms and effects. Int. J. Res. Pharm. Biomed. Sci., **3**(2):819-823.

Srinivasan, K.; Viswanad, B.; Asrat, L.; Kaul, C. L. and Ramarao, P. J. P. R. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res., **52**(4):313-320.

Surwit, R. S.; Kuhn, C. M.; Cochrane, C.; McCubbin, J. A. and Feinglos, M. N. (1988). Diet-induced type II diabetes in C57BL/6J mice. Diabetes, **37**(9): 1163-1167.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res., **50**(6):537-546.

Thayer, T. C.; Wilson, S. B. and Mathews, C. E. (2010). Use of nonobese diabetic mice to understand human type-1 diabetes. Endocrinol. Metab. Clin., **39**(3):541-561.

Todd, J. A. (2016). Intolerable secretion and diabetes in tolerant transgenic mice, revisited. Nature Genetics, **48**(5):476-477.

Tourrel, C.; Bailbe, D.;Meile, M.J.;Kergoat, M. and Portha, B. (2001). Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. Diabetes, **50**:1562-1570.

Ventura-Sobrevilla, J.; Boone-Villa, V. D.; Aguilar, C. N.; Román-Ramos, R.; Vega-Avila, E.; Campos-Sepúlveda, E. and Alarcón-Aguilar, F. (2011). Effect of varying dose and administration of streptozotocin on blood sugar in male CD1 mice. In: Proc. West. Pharmacol. Soc., **54**(5):9.

Wang, B.; Chandrasekera, P. C. and Pippin, J. J. (2014). Leptin - and leptin receptor-deficient rodent models: Relevance for human type 2 diabetes. Curr. Diabetes Rev., **10**(2):131-145.

Wang, Y. W.; Sun, G. D.; Sun, J.; Liu, S. J.; Wang, J. and Xu, X. H. (2013). Spontaneous type 2 diabetic rodent models. J. Diabetes Res., 401723.

Wang, R. N.; Bouwens, L. and Klöppel, G. (1996). Beta-cell growth in adolescent and adult rats treated with streptozotocin during the neonatal period. Diabetologia, 39:548-557.

Weir, G. C.;Clore, E. T.;Zmachinski, C. J. and Bonner-Weir, S. (1981). Islet secretion in a new experimental model for non-insulin-dependent diabetes. Diabetes, **30**(7):590-595.

Winzell, M. S. and Ahrén, B. (2004). The high-fat diet-fed mouse: A model for studying mechanisms and treatment of impaired Glucose tolerance and type 2 diabetes. Diabetes, 53(suppl_3),S215-S219.

Wu, J. and Yan, L. J. (2015). Streptozotocin-induced Type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes, metabolic syndrome and obesity: Targets and Therapy, pp:181-188.

Citation **Suwashni Priya, Mohd. Ibrahim Khan, Saif Mohammed Saleh Ansari, Mohammad Taha Kazmi and Danish Ahmed (2023). Neonatal streptozotocin induced diabetic rat models: A superior approach to mimicking human type 2 diabetes. J. Phytonanotech. Pharmaceut. Sci., 4(2):1-8. http://dx.doi.org/10.54085/jpps.2024.4.2.1**