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Streptozotocin Induced Diabetes in Animal Models

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ABSTRACT

In today's era**,** a simplified review plays a major role in better understanding of the experiment to be conducted. The major dictum of the review was to understand various aspects of gold standard diabetes-inducing agent Streptozotocin (STZ). This article covers History, Physical & Chemical Characteristics of STZ. The mechanism to induce Diabetes Role of 5-10% Glucose solution Induction of Type 1 & Type 2 Diabetes by using Streptozotocin Study of various diabetic complications.

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INTRODUCTION

Indeed Streptozotocin (STZ) is a naturally occurring alkylating antineoplastic agent that is particularly toxic to the insulin-producing beta islets of Langerhans cells of the pancreas in mammals. It is used as a drug for treating certain malignancies of the beta islets and used in pharmaceutical research to produce an animal model for hyperglycemia of type 2 diabetes or as well as type 1 diabetes with exclusive large or numerous low doses.

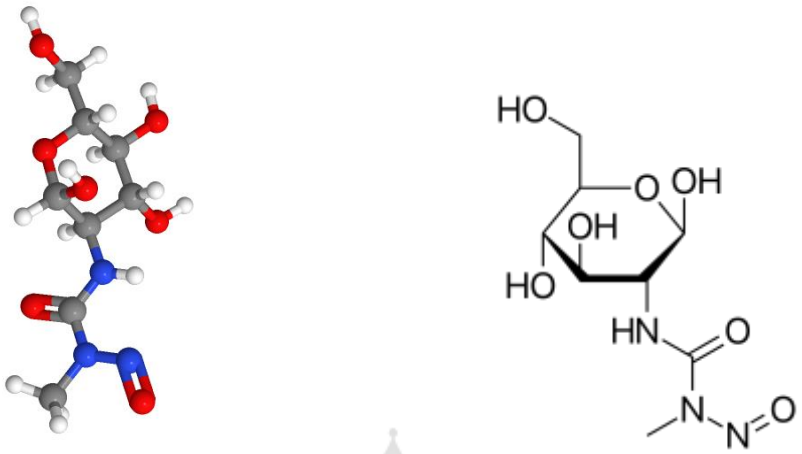

Figure no 01 Streptozotocin chemical structure and 3D model.

IUPAC name:- 2-Deoxy-2-({[methyl(nitroso)amino]carbonyl}amino)-β-D-glucopyranose.

Its Trade name is Zanosar. Route of administration for the treatment of malignancy is Intravenous. In animal models, it is mostly given by intraperitoneal route & mainly metabolism takes place in Liver & kidney. IUPAC name for STZ is 2-Deoxy-2-({[methyl(nitroso)amino]carbonyl}amino)-β-D-glucopyranose. C8H15N3O7 is Chemical Formula for STZ and physical Molar mass was found to be 265.221 g/mol.

History

JJ VAVRA et, al. identified STZ as a new antibacterial antibiotic in the late 1960s. [1] The medicine was discovered in a strain of the soil microbe *Streptomyces achromogenes* by researchers at the drug syndicate Upjohn Pvt Ltd.(now share of Pfizer) in Kalamazoo, Michigan. The topsoil sample in which the microbe turned up had been taken from Blue Rapids, Kansas, which can, therefore, be considered the origin of STZ. Upjohn company walk in a single file for patent protection for the medicine in August 1958 and U.S. Patent 3,027,300 was approved at the end of March 1962.

In the mid-1960s, streptozotocin was established scientifically to be selectively toxic to the beta cells of the pancreatic islets, the cells that normally control blood glucose levels by producing the hormone insulin. This advocated that the drug can be used as an animal model of diabetes[2-3] and as a medical treatment for malignant islet-cell tumor of the beta cells.[4] Mansford et, al. in 1968 found that when STZ administered to rats (65 mg. per kg. intravenously), gives rise to diabetes characterized by a specific hyperglycæmia with virtually no ketosis or elevation of plasma-free-fatty-acid concentration. Perfused hearts from streptozotocin-treated rats have normal concentrations of glycolytic intermediates, and the glycogen and citrate contents are not increased. [2] All of these results are in striking contrast to the findings in alloxan-induced diabetes. [2] In the 1960s and 1970s, the National Cancer Institute investigated streptozotocin's use in cancer chemotherapy. Upjohn company in July 1982 was granted approval by FDA which was filed for use of STZ as a cure for pancreatic islet cell cancer in November 1976. The drug was subsequently marketed as Zanosar. Streptozotocin is now available in many generic formulations. The basic need of this article is to gain knowledge of streptozotocin its pros & cons. This article will provide light on the various aspects of the STZ from A- Z that is from hyperglycemia to diabetic complications.

Table no 1: Table showing characteristics of Streptozotocin

Particular	**Description**
Structure IUPAC name	2-Deoxy-2-({[methyl(nitroso)amino] carbonyl}amino)-β-D-glucopyranose.
Source	streptomyces achromogenes
Appearance	Pale yellow or off-white crystalline powder
Solubility	Very soluble in water, ketones and lower alcohols, but slightly soluble in polar organic solvents.[5]
Molecular formula	C8H15N3O7 [6]
Molecular weight	265 g/mol [6]
Stability	Relatively stable at pH 7.4 and 37°C at least for up to 1 hr.[7]
Biological half-life	5–15 minutes [8,9]
Animals Used	Rats, Mice, Hamsters, Monkeys, Rabbits and Guinea pigs.

The mechanism to induce Diabetes

To know about the mechanism of STZ we must know the mechanism of Insulin Secretion.

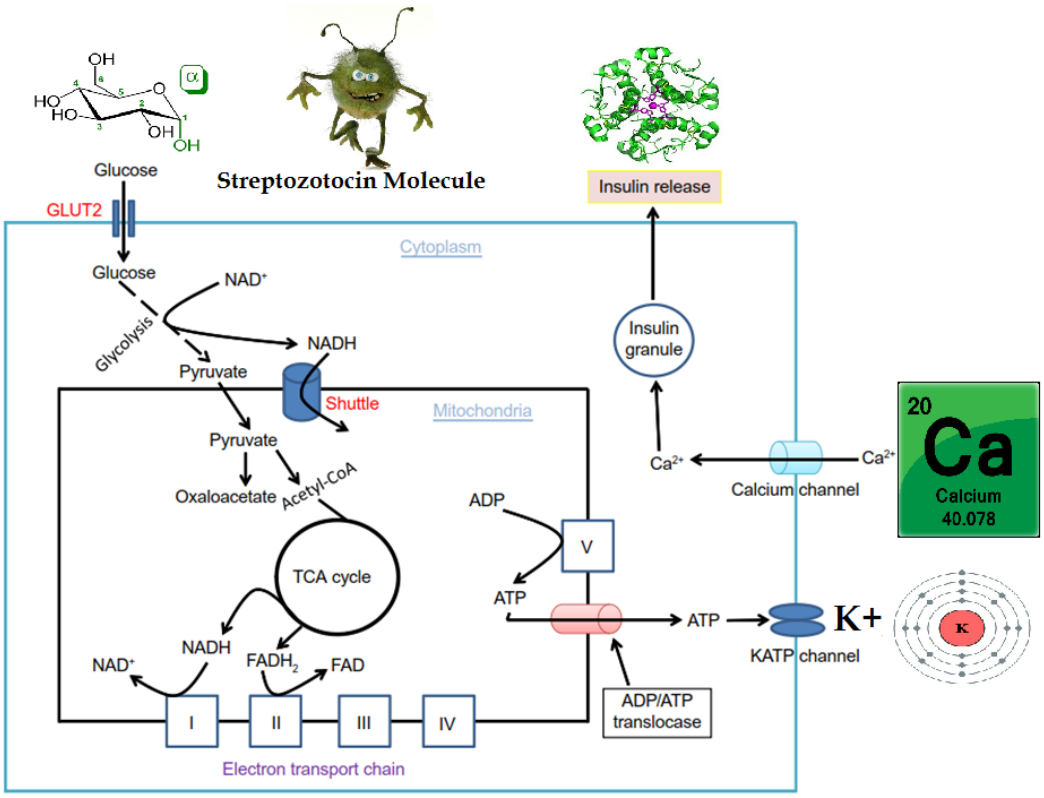

Figure no.-02. Streptozotocin is an evil analog of Glucose. Glucose combustion is tightly coupled to insulin secretion in pancreatic β cells. Notes: The figure shown depicts the main pathways of glucose metabolism and mitochondrial ATP production. Glucose is first transported into β cells via GLUT2 transporters, followed by glycolysis, Krebs cycle, and oxidative phosphorylation that eventually make ATP from the combustion of glucose. The elevated ratio of ATP/ADP, driven by high blood glucose, closes the KATP channel and opens the calcium channel on the cell membranes. The influx of calcium triggers the exocytosis of insulin granules and subsequent insulin release.

Rakieten L testified Studies on the diabetogenic action of Streptozotocin [10]. From that time STZ has been one of the diabetic inducers in experimental animals [11]. Streptozotocin functions as an alkylating agent & so DNA synthesis inhibitor in both bacterial and mammalian cells [12]. Streptozotocin is cytotoxic to pancreatic β-cells and it induces persistent hyperglycemia within 72 hours after administration subject to the dose administered strain & other factors[13]. The selective pancreatic beta islets cell toxicity and diabetic circumstance, resulting from STZ are connected to the glucose moiety. Its chemical structure which enables STZ to enter the pancreatic beta-cell via the low-affinity GLUT2 (glucose 2 transporters) in the plasma membrane[14] because the β-cells of the pancreas are

more active than other cells in taking up glucose moiety and so are more sensitive than other cells to STZ encounter. Elsner M et, al. validated this statement by the observation that insulin-producing cells that do not express this glucose transporter are unaffected to STZ toxicity [14] and only become susceptible to the toxicity of this compound after expression of the GLUT 2 transporter protein in the plasma membrane [14]. Furthermore, former cells that express this transporter such as the hepatocytes and the renal tubular cells are also vulnerable to STZ. This elucidates why experimental animals tend to have renal and liver damage[15]. After STZ challenge to non-beta cells such as α-cells as well as the extra-pancreatic parenchyma remains intact which indicate that beta islet cell-selective characteristic of STZ [16]. Due to the production of oxidative stress, STZ also causes inflammation, endothelial dysfunction cardiac and adipose tissue damage and [17] with the concentrations of the drug or its metabolites in the pancreas, intestine, kidney, and liver, being consistently higher than those in the blood plasma.

STZ is a structural correspondent of glucose (Glu) and N -acetyl glucosamine (GlcNAc).

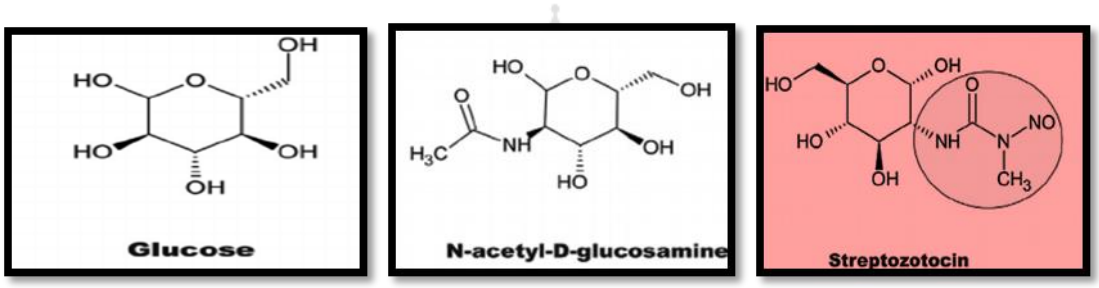

Figure no 3 Chemical structures of glucose, N-acetyl-D-glucosamine, streptozotocin.[18]

STZ is taken up by pancreatic β-cells via the GLUT 2 transporter where it grounds β-cell death by DNA crumbling due to the nitrosourea moiety. 3 key pathways linked with cell death are: **Fig 2.** (i) Free radical generation as hydrogen peroxide(H_2O_2) (ii) Nitric oxide (NO) production (iii) DNA methylation by the formation of CH3+ (carbonium ion) resulting in the stimulation of the nuclear enzyme poly ADP-ribose synthetase as chunk of the cell repair mechanism and thus, NAD+ depletion; [19,20].

Reactive Oxygen Species (ROS) Production in Oxidative Stress

Oxidative stress is well-defined as when there is a disparity between the pro-oxidants & antioxidant shield of the body as a result of steady-state reactive oxygen species. Oxidative stress has recently been exposed to be accountable, at least in part, for pancreatic β-cell dysfunction initiated by glucose toxicity in hyperglycemia. Numerous reaction mechanisms are thought to be involved in the origin of oxidative stress in both diabetic patients and diabetic animals and they comprise polyol pathway, advanced glycation end products, the formation of protein glycation and the glucose auto-oxidation [21,22]. During these processes, Reactive Oxygen Species are shaped and cause tissue impairment [23,24]. STZ action originates noteworthy increase in malonaldehyde but drops the number of antioxidant enzymes such as superoxide dismutase, glutathione, peroxidase, and catalase functions when compared with control animals in investigations. Which causes reductions in antioxidant function, and concurrently increases in malonaldehyde (MDA) activities, depicts the susceptibility of the pancreas to STZ's generation of oxidative stress [25,26]. One vital involvement of ROS during STZ metabolism is the production of uric acid as the concluding product of ATP degradation by xanthine oxidase using hypoxanthine. This reaction generates ROS such as hydroxyl radicals and superoxide originating from H2O2 dismutation during hypoxanthine breakdown, hastening the process of beta islet cell devastation. This is coupled with the fact that the pancreatic beta islet cells lack the glutathione peroxidase and catalase. The hydrogen peroxide consequently generates free radicals such as OH- and O2-. These over-reactive moieties are root cause of peroxidation of lipids, resulting in the formation of endoperoxides and hydroperoxy fatty acids. It upsurges the development of malonaldehyde and thromboxane-B2 (TxB2). The accretion of TxB2 along with thromboxane-A2 (TxA2) can lead to platelet aggregation and endorse thrombosis [28]. Augmented ROS production has also been testified to inhibit enzyme aconitase which guards mitochondrial DNA (mtDNA) from dilapidation [27].

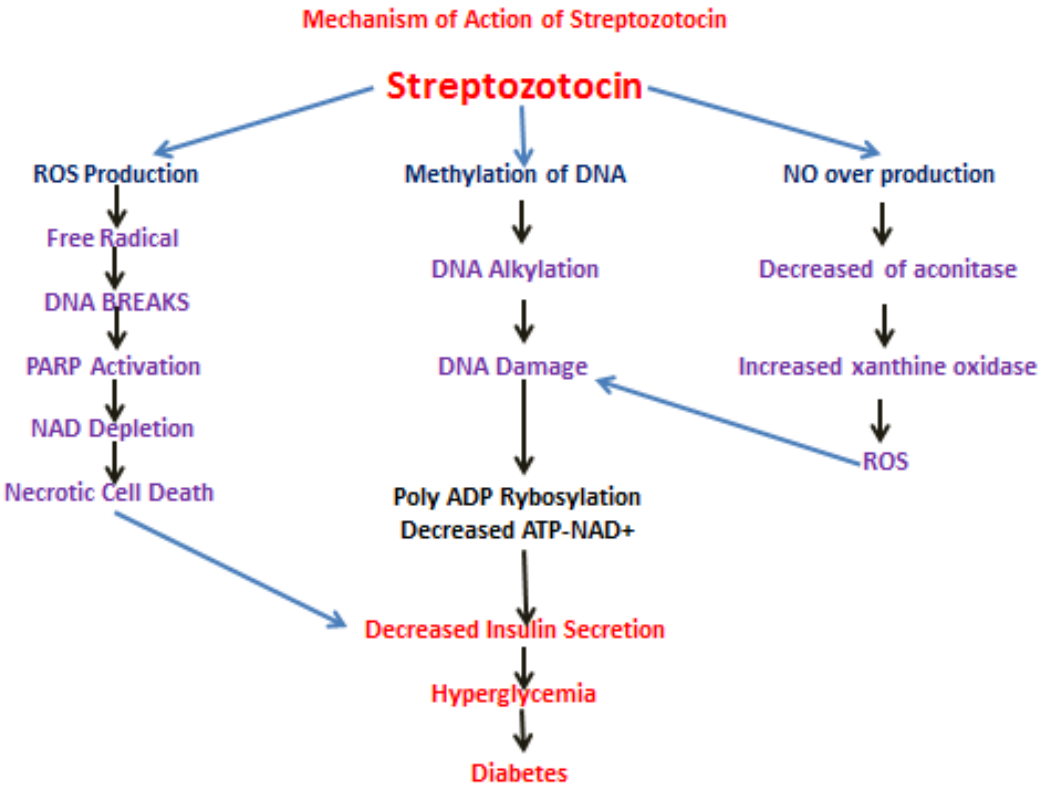

Figure no 3. (i) Free radical generation as hydrogen peroxide(H_2O_2) (ii) Nitric oxide (NO) production (iii) DNA methylation by the formation of CH3+ (carbonium ion) resulting in the stimulation of the nuclear enzyme poly ADP-ribose synthetase as chunk of the cell repair mechanism and thus, NAD+ depletion;

Nitric oxide (NO) overproduction

Additional likely mechanism of the diabetogenic exploit of streptozotocin that fallouts in cell death have been credited to its capability to act as nitric oxide contributor in pancreatic cells [29] which inhibits aconitase activity, leading to DNA alkylation and impairment [30]. Streptozotocin has been publicized to intensification the activity of guanylate cyclase and the development of cGMP, which are distinctive characteristic actions of NO. β- islet cells are mainly sensitive to injury by free radicals and nitric oxide as of their stumpy levels of free radical scavenging enzymes [31].

Alkylation of DNA

The DNA alkylating activity of the methyl nitrosourea moiety of STZ [32], especially at the O6 position of guanine, leading to DNA damage with subsequent necrosis of the pancreatic beta islet cells, through the exhaustion of cellular energy stores, is clarification for the cell

expiry that outcomes from STZ induction. The resulting stimulation of poly ADP-ribose polymerase (PARP), in an attempt to repair the damaged DNA, depletes the cellular NAD+ and consequently, ATP stores as a result of overstimulation of DNA repair mechanisms [33]. Although STZ also alkylates proteins, this DNA alkylation is most responsible for beta cell death, yet STZ alkylation of proteins could also donate to its toxicity to the beta-pancreatic cells. In totaling, inhibitors of this poly ADP ribose polymerase such as nicotinamide, inhibit the alkylation of DNA from STZ.

The practical approach of using Streptozotocin in an investigational animal model.

As compare to alloxan, STZ had demonstrated to be a better diabetogenic agent due to extensive species effectiveness and greater reproducibility. This could be credited to the datum that STZ is steadier in solution. Alloxan decreases hepatic glycogen within 24–72 hours, with superior cytotoxicity due to its conversion to anionic radicals [34] and pancreatic destruction. Furthermore, the STZ model emulates numerous of the acute and chronic complications of human diabetes and established the resemblances of some of the structural, functional and biochemical anomalies to human disease, it is a proper model to conduct the mechanism of diabetes. STZ must be protected from sunlight & must be administered within 12 minutes after dissolution.

Many investigators conclude that STZ produces type I diabetes mellitus [35]. The type of diabetes induced by STZ is controversial because STZ-hyperglycemia can be similar to either type I or type II diabetes mellitus [36, 37].

Table 2. Various Animals used as model for STZ induced diabetes.

Species	Dose	Route of Administration
Rats	50 to 75 mg/kg	IP(intraperitoneal)[38,39]
Mice	175 to 200 mg/kg	ip or iv (intravenous)[40,41,42]
Hamsters	50 -65mg/kg body wt.	ip(intraperitoneal)[43,44]
Dogs	15 mg/kg for 3 days	iv (intravenous)[45,46,47]

Citrate Buffer Preparation

Usually for rats pH-4.5 is preferred. To prepare 100ml of 0.1M citrate buffer....mix 44.5ml of the 0.1M citric acid monohydrate and 55.5ml of 0.1M trisodium citrate. dihydrate (pH-4.5)

- For 0.1 M sodium citrate (MW: 294.1) solution, dissolve 29.4 mg/ml in dH20.

That is dissolving 1.47 gm of Sodium citrate in 50 ml of distilled water. Adjust the pH to 4.5 with citric acid (Do not use HCL) Prepare freshly and keep it cold, before dissolving STZ.

- Some researchers find practically that the usage of citrate buffer for induction of diabetes is not necessary if you dissolve the STZ in cold normal saline solution induces diabetes too. But the amount of STZ which induces diabetes was very diverse in articles. In our experiment, it is 200 mg/Kg single dose for mice and 60-65mg/Kg single dose for rats was working well when you use the pure form you need a lesser dose.

- The stability of STZ in citrate buffer as well as in cold saline solution was also studied by various researchers. In both, the case stability is there, but u needs to prepare a fresh solution.

- Also, some people suggest that you need to filter sterilize the STZ solution through a 20 or 22um filter before injection into the animal. If not, you may introduce infections into the animal.

- Remove the food from mice/Rat (DO NOT remove the water bottle) and fast them for 6 hrs. and finally, formula is -

- **0.1 M sodium citrate**

Dissolve 1.47 g of sodium citrate tribasic dihydrate (MW 294.10) in 50 ml ddH_2O. and store at room temperature.

- **0.1 M citric acid**

Dissolve 1.05 g of citric acid monohydrate (MW 210.14) in 50 ml ddH_2O.

- Rather than using citrate buffer, some researchers suggested we can use $1x10^{-4}$N HCl, which will have a pH of ~ 4. STZ dissolves at this pH quite readily. The advantage of using HCl is that it has no buffering capacity and consequently the very little possibility of systemic alteration in pH even when you use as much as 4ml/kg. But it is better to stay at or below 2ml/kg. The key factor is it should be injected as quickly as possible.

Role of 5-10% Glucose solution.

One day/after 6 hrs. of glucose feeding after a single dose of STZ administration is absolutely crucial. The reason is pretty simple and straight forward. STZ has high toxicity towards beta cells, very specific too due to its entry through glut receptor of beta cells. Once inside, it causes DNA damage and NAD+ depletion, resulting in rapid necrotic cell death. This is associated with the release of massive amounts of preformed insulin, which could cause hypoglycaemic death. Administration of 5% glucose solution during the first 24 hours following STZ injection has been reported to prevent early mortalities [48,49].

Give 5-10% Glucose solution only after 6 hrs. of STZ Injection. Don't give food and glucose immediately because when people have tried giving 20% glucose feeding immediately after dosing with STZ it reversed the STZ action in rats there was the absence of diabetes. To overcome this glucose will be administrated after 6 hrs. of STZ post-administration. It might be due to there are multiple factors that can influence the ability of STZ to destroy beta cells, including strain-dependent variations. In this case you cannot expect re-emergence of beta cells after they are destroyed; however, we have to be mindful that STZ uses Glut-2 (high Km, ~16mM) to gain entry into beta cells just as doe's glucose. As It's better to know that STZ is a glucose structural analog but the beta cell GLUT2 is more sensitive to glucose than the STZ and if they are administered simultaneously, Glucose can prevent the diabetogenic action of STZ. In that context, we have to consider the possibility that by giving 20% glucose it may have sufficiently swamped the transporter by a large excess of a competing glucose molecule and considerably reduced the effective concentration of STZ that entered beta cells. If rats are fasted before treating them with STZ and waited for ~6hr before giving them glucose water, it is less likely that glucose had much of an effect on the action of STZ.

Induction of Type 1 & Type 2 Diabetes by using Streptozotocin

Many types of research have studied this phenomenon by using alone or in combination with other chemicals or with dietary simple manipulations & adding/ dose combining for induction of either type 1 or type 2 diabetes. [50,51].

Table no 3 Induction of Type 1 & Type 2 Diabetes by using Streptozotocin.

Type of Diabetes	STZ Dose with other	Description
Type 1	Single STZ	Depend upon the animal to used refer
Type 2	High-fat diet + STZ	Feed animals high-fat diet (HFD) (58% calories as fat) for 2 weeks after this Streptozotocin Injection is given n low dose.[58,59,61]
Type 2	Nicotinamide + STZ	Nicotinamide (95 mg/kg b.w.) (dissolved in saline, was injected intraperitoneally 15 min before administration of STZ.[65,67,68]
Type 2	Neonatal period + STZ	By injecting Wistar rats on the day of their birth (n0=birth) intravenously (saphenous vein) or intraperitoneally with 100 mg/kg of STZ.[62,63] or spontaneously hypertensive rats were treated with 25.0, 37.5, 50.0, 62.5 or 75.0 mg/kg of streptozotocin given intraperitoneally at 2 days of age and maintained for 12 weeks.[64,67]
Type 2	High-fat diet + Nicotinamide +STZ	Rats were fed five-week high-fat diet and then received intraperitoneally then after 5 weeks Nicotinamide (230 mg/kg) followed by STZ (65 mg/kg)intraperitoneally.[66]

Type 1 diabetes can be tempted in rodents by a sole STZ injection[52,53] while type 2 diabetes can be induced by at three lines, which comprise of STZ injection after 1) administration of nicotinamide, [54,55] high fat diet (HFD) feeding followed by a low-dose STZ injection,[56,59,60,61] and STZ injection during the neonatal period.[57,58,62,63,64]. These various models can be used in elucidating the mechanisms of diabetic pathogenesis and in screening medicines, drugs, artificial chemicals, synthetic compounds, lead moieties, natural products, and pharmacological agents that are effective in the management of diabetes. High-fat diet followed by moderate i.v STZ dose (35 mg/kg) in 24 h fasted rats is preferable than the single high dose (70 mg/kg) to induce stable hyperglycemia with minimal or no mortality. Nicotinamide given at a dose of 230 mg/kg i.p. or orally before 15 min of STZ administration (65 mg/kg, i.v.) yields substantial stable hyperglycemia with maximum

survival of animals and the extent of b cell protection by nicotinamide suddenly decreases when the interval between STZ and nicotinamide injections exceeds 1 h. Administration of L-glutamine for 4 months from the 15th day of administration of STZ may prevent STZ induced cardiomyopathy. Reproductive toxicity of STZ can be protected by administering 1000 mg/kg dose of D-Glucose divided into two doses with an interval of 2 min and STZ administered in between the interval without causing any effect to the diabetogenic action of STZ. [69]

Study of various diabetic complications

Diabetic complication	Description
Diabetic Neuropathy	RAT:-65-75mg/kg IP Up to 4 weeks post STZ dose.[70,71,72,73,74,75] MICE:- 100-200mg/kg IP Up to 4 weeks post STZ dose.
Diabetic Cardiomyopathy	Single STZ dose and after 4weeks it was found to be developed. Treatment was continued until 8 weeks.[76,77,78,79]
Diabetic Nephropathy	Single STZ dose and after 3 weeks it developed to test drug is given up to 8 weeks. [80,81,82,83,84,85]
Diabetic Immunopathy	STZ is not suitable to study immunology related diabetic complications as it has a direct toxic and depressive effect on immune cells.[86,87] Here Alloxan is best suited as it produces diabetes in a more natural way as well as diabetic Immunocomplications.[88,93,94]
Diabetic Wound Healing	Single STZ dose and after 10 days it developed to test drug is given up to 21days. (Till wound clears) [89,90,91,]
Diabetic Hepatopathy	Rat:- Generally after 20 days hepatopathy develops and treatment is given up to 56 days.[95,96,98] Mice:-9 week to induce diabetes and up to 12-week treatment is given. Most preferably in the mice model High-Fat diet Type 2, Diabetes is done.[97,98,99,100]
Diabetic Sexual Problems	Single STZ dose and after 3weeks it was found to be developed. Treatment was continued until 6 weeks. In rat female vaginal properties were studied upto 12 weeks.[101,102,103,]

CONCLUSION

From the above data, we can suggest that if the cost is not an issue then streptozotocin will be the best model to study diabetes and its related complications. Except for diabetic Immunological complications due to the direct toxic effect of STZ.

No conflict of interest.

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