OPTIMIZATION OF PROTOCOL FOR THE SUCCESSFUL INDUCTION OF STREPTOZOTOCIN-INDUCED EXPERIMENTAL TYPE 2 DIABETS IN RATS

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Diabetes mellitus (DM) is a multifactorial, multisystemic endocrine disorder in which the living body does not produce (type 1) and/or properly respond (type 2) to insulin, a pro-hormone essential for the entry of glucose from the plasma to cells for the generation of energy. Despite persistent elevation in blood glucose levels, the insulin dependent cells are deprived of glucose and other pathways capable of producing glucose such as gluconeogenesis start to predominate. Thus, diabetes affects nearly every organ system in the body and has become a serious public health problem in the world. Chronic hyperglycemia in diabetes is an established risk factor for the development of micro as well as marcro vascular complications and premature mortality.

The pathogenesis of diabetes mellitus and its management by the oral administration of hypoglycemic agents have stimulated great interest in recent years. The currently prescribed drugs having different mechanisms of action for the treatment of diabetes and its secondary complications time and again possess characteristic profiles of side effects apart from the development of drug resistance. The existence of experimental animal models facilitates in the understanding of the pathophysiology of diabetes and its secondary complications due to chronic hyperglycemia and aids the development of novel drugs with maximum efficacy without side effects. In the present review, the protocols used for the successful induction of experimental diabetes are described with suitable references for easy understanding.

Significant characteristic features of T2DM mainly include insulin resistance and the failure of pancreatic β cells to compensate for this resistance. Consequently, insulin-resistant and/or animal models with β-cell failure are often preferred for T2DM research. Obesity is frequently observed in animal models of T2DM, reflecting the human condition in which obesity triggers insulin resistance, a process directly linked to the initiation, progression, and onset of T2DM. Insulin facilitates the transfer of blood glucose into body cells, except for those in the brain, active muscles, and liver. When glucose cannot enter body cells to generate energy in the form of ATP for vital cellular functions, it remains in the plasma and leads to chronic hyperglycemia, which results in several secondary complications. Adipokines such as resistin and adiponectin result in impaired insulin action in muscle [1].

Several methods have been employed to induce T2DM in laboratory animals with inconsistent success and many hitches. Rodent models of diabetes can be categorised as genetic and experimentally induced. The most commonly used animal models of T2DM include the spontaneous or genetically derived animal models available, such as the Zucker diabetic fatty rat (ZDF), the Goto-Kakizaki rat (GK), the Ousuka Long Evans Tokushima fatty (OLETF) rat and the db/db mouse, which exhibit obesity characteristics, insulin resistance and impaired β-cell function. Even though these animal models have contributed significantly to the understanding of the pathophysiology, complications and treatment of diabetes, these animal models are costly and are not readily available for the detailed experimental purpose.

Experimentally induced animal models of diabetes are produced by surgery, dietary manipulation, diabetogenic chemicals or their combination. An animal model is a living

being in which an observable fact of interest, similar in several characteristic features to humans, is studied in a way that cannot be studied in humans. The results generated through animal models are widely used for building conceptual models to generate verifiable hypotheses to extrapolate in humans [2].. Using animal models in biomedical research has a long history [3]. An estimate indicates that the number of experimental animal models used for research purposes was well over 125 million per year and is still increasing [4].

A report on toxicological studies indicates that the concordance between adverse findings in clinical data with the data generated in experimental animal models was around 71% [5]. In addition, according to the Nuremberg Code [6], which is considered the cornerstone for conducting ethical biomedical research, animal studies need to be conducted before human clinical trials [7]). Despite the several limitations, experimental animal models have remained the best alternative tool for testing hypotheses before human trials and a core of preclinical drug development, which is a lengthy and expensive process [8]. Each animal model, provides many advantages for understanding the pathophysiology of some areas of the disease [9]. By choosing the appropriate animal model, researchers can reveal, assess, and evaluate the antidiabetic efficacy of various medications or newly developed agents.

Rats and mice are the preferential animal models for biomedical research, especially diabetes. Diabetic research in rats featured in 95% of articles in the field of endocrinology, and this is mainly due to the accessibility, availability and short generation interval [10, 11]. Further, the rat model of diabetes is more similar to human diabetes, for example, in the ability of agents to modify the disease, in addition to the fact that the rats have a short gestation period of 21-22 days and reach sexual maturity at post-natal days of 60-70 days [12, 13]. However, the relatively larger size of rats compared to mice facilitates serial blood sampling more easily. Rats are widely used to understand the pathophysiology of diabetes, including the causative factors related to the disease, symptoms, evolution and secondary complications. They have also been often used in the development of effective drugs for its treatment. The onset of T2DM is gradual and includes a functional insulin deficiency and sensitivity, which are known to be directly linked to ageing or obesity. In most cases, individuals with T2DM have a family history with the same disease or other medical problems which are related to diabetes, such as high cholesterol levels, high blood pressure, and obesity. In most cases, individuals with T2DM have a family history with the same disease or other medical problems which are related to diabetes, such as high cholesterol levels, high blood pressure, and obesity.

Chemical-induced experimental T2DM offers a rapid and relatively inexpensive way to simulate diabetes in rodents. Chemicals used for the induction of diabetes in animal models include alloxan, streptozotocin, dexamethasone, bovine-insulin-Freund’s adjuvant, vector, dithizone, 8-hydroxyquinolone, monosodium glutamate and gold thioglucose [14, 15]. Chemicals that induce diabetes in rodents can be divided into three categories: (1) those that specifically destroy the insulin-producing β cells of the pancreas. (2) those that temporarily restrict insulin synthesis and/or secretion, and (3) those that reduce insulin’s effectiveness in target tissues such as the liver. However, the chemicals belonging to the first category are

often preferred because of the fact that they cause lesions and other pathological features similar to human diabetes. Among the various chemicals, either alloxan or streptozotocin is the most well-known diabetogenic chemical in diabetes research and has been widely used to induce diabetes in animal models. Although their cytotoxicity is achieved via different pathways, their mechanisms of β-cell damage selective action are similar. Alloxan (ALX) causes the β-cell toxicity through the generation of excessive reactive oxygen species (ROS), whereas in the case of streptozotocin (STZ), the action is through deoxyribonucleic acid (DNA).

Due to the relative instability of these chemicals, the solutions should be ideally being prepared just before injection. Fasting animals are typically more susceptible because of the fact that these chemicals can compete with glucose due to their structural similarity [16]. In addition, these chemicals might be hazardous to other body organs, and hence they should be handled carefully. Additionally, it is essential to note that the administration of these chemicals has been linked to alterations in P450 isoenzymes in the liver, kidney, brain, intestine, lung, and testis, and hence their effect has to be considered when the efficacy of drugs is evaluated in these models [17] .Due to unique chemical properties, STZ is the chemical agent of priority to develop a diabetic metabolic state in experimental animals, as it relatively has greater stability while alloxan is appropriate upon the understanding of the ROS-mediated mechanisms of β-cell death in diabetes mellitus [18].

Alloxan (2, 4, 5, 6-tetraoxypyrimidine: 5, 6-dioxyuracil) is a well-known diabetogenic agent used to induce diabetes in experimental animals such as rats, mice, rabbits and dogs, with the rabbit being less so [19, 20]. It is chemically classified as a pyrimidine derivative. Alloxan-induced experimental diabetes in laboratory animals is easy to perform and cost-effective. Alloxan is converted to dialuric acid and then re-oxidised back to its original state, creating a cycle of superoxide radicals that cause hydrogen peroxide and highly reactive hydroxyl radicals capable of breaking down the β-cell DNA [21]. Oxidation of protein -SH groups is one of the other mechanisms of β-cell damage by alloxan, which affects intracellular calcium homeostasis [22]. Depending upon the animal species, the dose and route of administration of alloxan in the form of alloxan monohydrate were determined. In mice, alloxan monohydrate is injected subcutaneously at a dose of 50-200 mg/kg, and in Wistar or Sprague-Dawley rats weighing around 150-200 gm, the dose is equal to 150-175 mg/kg.

In rabbits weighing 2.5-4 kg, during a course of 10 min, alloxan monohydrate at a concentration of 5 g/100 ml, pH 4.5, is infused via the marginal ear vein (150 mg/kg). In male dogs weighing 15-20 kg, intravenous injection with 60 mg/kg of alloxan monohydrate is commonly applied. In non-human primates such as monkeys and baboons, alloxan monohydrate is injected intravenously at a dose of 65-200 mg/kg to induce diabetes. Depending upon the researcher's skill and proper preparation of the alloxan monohydrate solution in the specified buffer, more than 70% of the animals will become hyperglycaemic and uricosuric after the administration of the alloxan in the form of injection. However, it is mandatory to treat the animals with glucose solution or insulin, if necessary, intravenously for one week along with food *ad libitum* to avoid severe hyperglycemia [23]. Residual insulin

secretion in alloxan-induced experimental diabetes plays a crucial role in the animals living a long time without insulin treatment [24].

Streptozotocin, chemically known as N-(methylnitrosocarbamoyl)-alpha-d-glucosamine, is a naturally occurring antibiotic compound produced by *Streptomycetes achromogenes and* was discovered in 1959. Rakieten first reported the use of STZ as a diabetogenic chemical [25]. Its toxicity towards pancreatic β-cells was due to its structure causing its destruction capacity [18, 26, 27]. The molecular formula of STZ is $C_8H_{15}N_3O_7$ with a molecular weight around 265 [28]. STZ is a pale yellow, freeze-dried powder [29] stored at -200°C [30]. Based on the first reports on the diabetogenic action of STZ, the solution of STZ is prepared and maintained in the ice-cold acidic citrate buffer at pH 4.5, and it is used immediately after its preparation [30, 31] .The STZ molecule has two parts: (1) a glucopyranosyl group, which facilitates its rapid uptake by the pancreatic β-cells by glucose transporter 2 (GLUT2), and (2) a nitrosourea group, which selectively destructs pancreatic β-cells. Studies in mice [32] humans [33] rats [34] indicate that maximum plasma concentrations of STZ are lower than predicted values, evidencing a rapid initial metabolism of STZ in plasma. In rodent β-cells, GLUT2 is the predominant glucose transporter [35].

The dose of STZ itself obviously has a significant impact on the phenotype of rats. STZ induces robust, not absolute, β-cell ablation in a manner that depends on the dose, number of doses, time interval between the doses, route of administration, fed / fasted state upon STZ administration and the rat strain. Glucose homeostasis [36] β-cell function [37], insulin sensitivity and type 2 diabetes are sex-dependent phenomena. Women have about 6% more β cells than men [38] and more severe adverse health consequences occur in women than men [39]. Impaired fasting glucose is more prevalent in men, and impaired glucose tolerance is more prevalent in women [40, 41, and 36]. This sex bias causes a generalisation of findings in men to women without appropriate justification [11]. Further, some reports suggest that researchers prefer male animals over females to lessen mortality and increase the efficacy of STZ [42, 43, and 44].

Additionally, the mass of β cells in both humans and rodents is dynamic [45]. The lifespan of rat β-cells is 1-3 months [46]. New pancreatic β-cells are formed by neogenesis through differentiation from undifferentiated precursors such as embryonic duct cells and also by replication from pre-existing differentiated cells. Pancreatic β-cell replication in rats is age-dependent; it is highest at birth, reaches about 3% by postnatal days 30-40 and remains constant after that. Susceptibility to the diabetogenic effect of STZ is inversely related to animal age [47], which in turn is related to decreased capacity for β-cell regeneration [48,49,50,51]. More than 1000 rat strains have been used for research purposes, and strain differentiation in the susceptibility to STZ has been reported [52-58]. However, Wistar and Sprague-Dawley rats are the most commonly used rat strains for STZ-induced diabetes due to their sensitivity to STZ [59].

Regarding the route of administration of STZ to experimental rats, intraperitoneal injection is preferred because it is easier, much more used and has a high reproducibility [60, 42, 61-63].

However, it has been found to be associated with the risk of intestinal injury and mortality, and its penetration into the subcutaneous tissue reduces its diabetogenic effect. Above all, the mortality was due to hypoglycaemia during the first 24 hrs or severe hyperglycaemia that occurred after 2-3 days of intraperitoneal administration of diabetogenic doses of STZ. It has been reported that those days 2 and 3 after STZ injection is the critical period regarding the mortality rate [64-67].

Early hypoglycaemia-related mortality is more pronounced in fasted animals and can be prevented by providing rat access to food soon after STZ injection or administration of 5% glucose during the first 24 hrs [68]. On the other hand, hyperglycaemia-related mortality following injection with diabetogenic doses of STZ can be overcome with insulin administration, and the dose of insulin required varies according to the severity of the hyperglycaemic index, species, and strain and should be determined by the researcher, but the dose should be a minimum dose of long-acting insulin [3,42,69] .Thus, it is necessary that the diabetes researchers need to have a better understanding of animal models of diabetes to derive robust outcomes that have more chance to extrapolate to humans.

The Glucose Transporter (GLUT) protein family belongs to the major facilitator superfamily of membrane transporters, comprising fourteen proteins, which typically transport sugars down concentration gradients [70]. They catalyse the facilitative (energy-independent) bidirectional transfer of their substrates across the cell membranes, and they may exhibit either symmetric or asymmetric transport kinetics. The glucose transport proteins are composed of about 500 amino acids and are predicted to possess 12 transmembrane-spanning alpha helices and a single N-linked oligosaccharide. Each GLUT isoform is expressed in a distinct tissue distribution. The transport involves two steps, namely, (1) glucose binds at the surface of the transporter, and (2) a conformational change moves it across the membrane and releases it.

GLUT 1 catalyses the rate-limiting step in the central nervous system (CNS) for the supply of glucose, and its ability to acutely upregulate its activity might be a very useful strategy to counter the effect of cardiac strokes due to arterial blockage as well as the damage that occurs to cardiomyocytes during cardiac infarction [71] .GLUT1 is frequently upregulated during oncogenesis in different tissue types, a process that is probably essential for tumours to grow beyond a size limited by their glycolytic ability [72].

GLUT2 is expressed at a very high level in pancreatic β-cells and reaches the apical surface in the presence of high luminal glucose concentration to increase the glucose absorption [73] .GLUT 3 is the major neuronal glucose transporter present in both dendrites and axons [74]. GLUT 4 is abundantly expressed in adipose tissue and skeletal muscle that facilitates the transport of hexoses across the cell membrane through the ATP-independent, facilitative diffusion mechanism [75-77]. GLUT5 is highly expressed in the apical membrane of intestinal epithelial cells [78]. GLUT6 was originally cloned from the leucocytes, and its primary physiological substrate has not been established [79]. GLUT7 is expressed in the apical membrane of the small as well as the large intestine [80]. Studies with knockout mice

indicate the role of GLUT8 indicates its role in hippocampal neuronal proliferation and heart atrial activity [81]. GLUT9 is present in the liver, kidney and intestine and at a lower level in chondrocytes, and its major function is linked to renal urate reabsorption [82]. Mutations in the GLUT10 gene are the cause of arterial tortuosity syndrome [83]. GLUT11 exists in three different forms that vary at their extreme amino terminal and those are expressed in different tissue types and exhibit glucose and fructose transport activity [84]. GLUT12 is involved in the overall regulation of glucose homeostasis. GLUT13 is a myoinositol transporter and also called HMIT. It is expressed primarily in the brain and is the only glucose transport protein that appears to function as a proton-coupled symporter. GLUT14 has a sequence similar to GLUT3, and it is expressed in testes [85].

In evidence of the hypothesis that the cytotoxic effect of STZ is associated with glucose transport capacity, rat insulinoma cell line cells, which do not express GLUT2 and express GLTU1 instead, show resistance against STZ toxicity [86]. Overexpressing GLTU2 in rat insulinoma cells causes STZ transportation with high affinity and thus, β-cell toxicity [87]. Glucose uptake in rat β-cells is mainly mediated through GLUT2 [35], which has a very high Vmax (32 mmol/min/L islet space) and a high Km (17 mM) for glucose [88] .The high transport capacity of GLUT2 causes rapid equilibrium between extracellular and intracellular glucose. In contrast, Km of glucokinase (4-10 mM), which phosphorylates glucose inside the β-cell, is much lower than GLUT2; in fact, glucose uptake in rat β-cells is about 66 and 88 times faster than glucose utilisation by glucokinase. These reports suggest that glucokinase-dependent glucose phosphorylation is the rate-limiting factor for glucose utilisation in β cells rather than GLUT2-mediated glucose uptake [89]. STZ is also a cytotoxic glucose analogue like alloxan [90].

Among the various chemicals used for the induction of diabetes in experimental animals, STZ is the most commonly used chemical [91]. STZ, the most prominent diabetogenic chemical, is widely employed in experimental animals for inducing animal models of both type 1 and type 2 diabetes [62,92]. In chemically induced models, either a single high dose or multiple sub-diabetogenic low doses of STZ administered for 5 consecutive days intraperitoneally or intravenously are used in rats. It has been suggested that the best index of the diabetogenic activity of STZ is the pancreatic insulin content 24 hr after its intravenous administration [93]. However, it has been reported that a 40 mg/kg dose of intraperitoneal injection is optimal for creating diabetes with hyperglycemia in Wistar rats of both sexes, which resembles most of the clinical features of human diabetes, to screen the efficacy of lead molecules and natural products [94]. The LD50 of STZ in rats is about 130 mg/kg [64] ,which is lower than that in mice, which is about 345 mg/kg [95].

STZ is a nitrosourea analogue in which the N-methyl-N-nitrosourea moiety is linked to the carbon-2 of a deoxyglucose that gets accumulated in the pancreatic β-cells through the GLU-2 transporter uptake [96, 97]. GLUT2 are abundantly expressed in the pancreatic β-cells and cause DNA alkylation followed by the activation of poly ADP ribosylation, leading to the depletion of cytosolic concentration of NAD+ and ATP. Enhanced ATP dephosphorylation after STZ administration provides substrate for xanthine oxidase, resulting

in the excessive generation of superoxide radicals. Further, nitric oxide moiety is liberated from STZ, leading to irreversible destruction of insulin-producing β cells by necrosis [21]. It was observed that STZ administration primarily abolished the β-cell response to blood glucose stimulation. Though temporary return to glucose responsiveness appears, it is followed by permanent loss of β-cell mass and its biological functions [98].

STZ administration induces diabetes in mice, rats and other animals like rabbits and guinea pigs in two ways depending on the dose [99]. Following intravenous or intraperitoneal administration, STZ targets pancreatic β-cells by the transmembrane carrier protein GLUT2 and causes DNA to be alkylated through its alkylating property, which is a normal function of the cytotoxic nitrosuria compounds [87]. In general, the nitrosuria compounds are lipophilic in nature and hence are easily taken up by the cells, but in contrast, the STZ, being a nitrosouria compound, is hydrophilic due to hexose substitution and not easily taken up by the cells; therefore, STZ is carried by a carrier protein of glucose known as GLUT-2 to the β-cells because the chemical structure of the STZ resembles the glucose moiety [100] .The β cells of the pancreas usually have selective properties of the STZ, and hence, it keeps the alpha cells of the pancreas and the extrapancreatic cells in an intact condition and does not affect them [101]. Additionally, STZ is a potential source of free radicals, which can also cause DNA damage and ultimately lead to β-cell death [31].

DNA fragmentation results from the transfer of the methyl group from STZ to the DNA molecule, which damages it through a specific chain of events [18]. Further, at low doses of administration, STZ induces an immune and inflammatory response which is due to the release of the enzyme glutamic acid decarboxylase [61]. This condition facilitates the selective destruction of β cells and leads to the development of chronic hyperglycemia that is associated with inflammatory infiltrates, in particular with lymphocytes of the pancreas [102].

STZ is a hydrophilic agent [18]A single high dose or several low doses of STZ dissolved in 0.1 M ice-cold citrate buffer, pH 4.5, over 5 days is delivered by intravenous injection to mice or rats to cause insulitis [103]. Intravenous or intraperitoneal administration of STZ to experimental animals at a concentration of 35-65 mg/kg often results in a triphasic response is observed in blood glucose concentration [66,68,98,]; (1) an early transient hyperglycemia within 2-4 hrs after STZ administration probably due to adrenaline response and sudden breakdown of liver glycogen without a partial increase in serum insulin levels; (2) transient hypoglycaemia within 7-10 hrs after STZ injection due to increased serum insulin levels because of insulin release from the necrotizing β-cells without a decrease in pancreatic insulin content and, (3) stable hyperglycemia 24hrs after STZ administration evidencing frank diabetes characterized by permanent elevation in fasting, postprandial and random blood glucose levels and relative insulinemia [98,104,105] .Initially, blood glucose increases to 150-200 mg% within 3 hr after treatment with STZ. There is an association between the dose of STZ administration and the diabetic state induced [106].

When compared to alloxan, the induction with STZ causes stable and reproducible diabetes [107]. It is often considered a simple and valuable tool to induce diabetes in several laboratory animals, generally in rats [108]. Above all, induction of diabetes by using STZ is convenient [109]. The dose of STZ should be optimized so that diabetes is successfully induced and, simultaneously, significant mortality can be avoided [110]. Mandatory factors that should be considered when a dose of STZ is used include animal age [49,111], sex [44,112,113], strain [54,57], and the route of administration of STZ [42]. Therefore, it is suggested that for producing stable and frank hyperglycemia, researchers should optimize the dose of STZ between 40 and 60 mg/kg by considering the above factors. Further, the duration of fasting is an important factor that can cause variations in clinical data during the experimental studies [114].

It is preferable to set a fasting duration of 16 hr for preclinical studies involving clinical pathology measurements in rats to minimize the competition between glucose and STZ for GLUT2-mediated uptake into the β-cell [31, 89,115]. Above all, the individual's skill is very much essential for the successful induction of diabetes in experimental animals.

Among the different models of experimentally induced type 2 diabetes in rats, STZ is used in high-fat-diet-fed and low-dose STZ [116-118], STZ-nicotinamide [119], and neonatal STZ [48]models. The High Fat Diet (HFD) was prepared indigenously by using a normal pellet diet, raw cholesterol, and a mixture of Vanaspati ghee and pure coconut oil (2:1). Briefly, the normal rat pellet diet was powdered by grinding and mixed with 2.5% cholesterol and a mixture of Vanaspati ghee and coconut oil (5%). The mixture was made into pellet form and orally fed to rats to induce metabolic syndrome [120]. The rats were divided into two dietary regimens by feeding either normal or HFD for the initial period of 2 weeks.

After 2 weeks of dietary management to develop insulin resistance, the groups of rats fed with HFD were intraperitoneally injected with a freshly prepared low dose of STZ (35 mg/kg b.w.) dissolved in 0.1M ice-cold citrate buffer, pH 4.5 [121]. Rats having the fasting blood glucose levels ≥300 mg/dl on the 3rd day after STZ injection were considered as diabetic and subjected to further studies.

In the STZ-Nicotinamide animal model, first introduced by [119], nicotinamide was administered intravenously 15 minutes before STZ injection to provide partial protection in β-cells; stable hyperglycemia, reduced pancreatic insulin content by 60%, and insulin responsiveness to glucose are among the main advantages of this model. Owing to the inhibitory potential on poly-ADP-ribose synthetase activity, the pharmacological doses of nicotinamide prior to STZ administration are meticulous in preventing the β-cell genotoxicant-mediated depletion of nicotinamide adenine dinucleotides and modulating the decline in the proinsulin quantity, thereby partially reversing the inhibition of insulin secretion to prevent the aggravation of experimental diabetes. This condition is epitomized by constant hyperglycemia, glucose intolerance, and significantly altered glucose-stimulated insulin secretion that contributes to a number of features similar to human diabetes. This

model further validated the use of the antidiabetic drug tolbutamide and consequently recommended its use for the pharmacological screening of new insulinotropic agents.

An additional advantage of this model, other than its specific application to T2DM, is the relatively short time it takes to develop diabetes [119]However, lack of insulin resistance is its major disadvantage. In neonatal STZ rats, STZ is injected on the day of birth, two days after birth, or five days after birth, which causes a more than 50% decrease in the number of pancreatic β cells. Due to high mortality associated with this model due to chronic hyperglycemia, it is not preferred by several researchers [14,31,118,122-124].

In summary, we conclude that the detailed protocols presented in this article will be of immense use for producing STZ-induced insulin deficiency and/or resistance with chronic hyperglycemia in experimental animals such as rats and mice. Though STZ is a valuable chemical for inducing diabetes in rats, some practical guidelines specified above should be considered to perform well-conducted and ethical animal research. However, a high-fat diet fed with a low dose of streptozotocin induced experimental type 2 diabetes in male rats of different strains and was effective, as it resembles most of the clinical features that are very similar to human type 2 diabetes mellitus in assessing the pathophysiological consequences of diabetes and for screening potential therapies for successful treatment. Finally, differences between animal modeling and humans should be considered, including differences in gene regulation, differences in life spans, metabolic regulations, and the purpose of the study to reveal, assess, and evaluate the antidiabetic efficacy of various medications or newly developed lead molecules.

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