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Chemical Induction of Diabetes in Rodents: A Critical Tool for Diabetes Research

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Abstract

Diabetes mellitus, a chronic ailment, frequently associated with permanent and irreversible functional and structural changes in the body cells, with around 422 million sufferers worldwide. Succour in the use of products having anti-diabetic activity of better performance with less toxicity and cost is being sorted. Researchers worldwide have long been encouraged to intensify efforts in the search for more effective treatment remedies, notwithstanding there exist already available hypoglycaemic agents and insulin. Many researchers are joining this research quest to avail for the best treatment agent so as to control the frequent disease corollaries. The experimental model of a disease aids the understanding of the pathophysiology of the disease and the development of drugs for its management. The ease of handling rodents makes their use most appropriate for research purposes. There are genetically engineered rodents, as well as other rodent models such as chemical (streptozotocin, alloxan, and Ferric nitrilotriacetate) which has gained much use amongst scientists in this hub because of affordability, availability, and ease with use, an advantage of low income researchers in resource poor economy (African institutions). The cumbersome effort in trying to decide on the method and chemical of choice in traversing this research area gave the need for a wide review of researches carried out spanning from 1975 to 2023. The criteria to be used forinclusiveness being: The frequency of use of the chemical; The most frequent dose and route of administration employed; The ease of the administration in terms of technique following the advanced search using these words ''chemical method of diabetes induction in rodents(NOT reviews)'' spanning from 1975 to September 2023. The search engines employed were Base; PubMed; Google scholar; World cat search and Science direct. The most frequently engaged chemical methods remains, streptozotocin and alloxan induction respectively. The preferred route/ dose for streptozotocin for chemical induction are Intraperitoneal/ 35- 65mg/kg; and Alloxan Intraperitoneal/ 70-170mg/kg.

Keywords: Streptozotocin; Alloxan; Induction; Diabetes

Abbreviations

STZ: Streptozotocin; ALX: Alloxan; IP: Intraperitoneal; IV: Intravenous; IM: Intramuscular.

Introduction

As at 1999, 140 million people suffered from diabetes mellitus worldwide [1]. In 2023, the figure stands at around 422 million people, the majority are found in low-and middle-income countries [2]. This figure already overshoots the predicted estimate of 438 million by 2030 as prevalence of diabetes [3]. Diabetes accounts for 1.5 million deaths directly every year [2]. It is a genuine conclusion therefore that the number of cases and the prevalence of the disease have steadily increased over the past decades.

Diabetes mellitus is a chronic group of multiple organ disorders with different aetiologies, a serious endocrine syndrome characterized by derangement of carbohydrate, protein, and fat metabolism caused by a complete or relative insufficiency in insulin secretion and/or insulin action, frequently associated with permanent and irreversible functional and structural changes in the body cells [4]. These frequent permanent and irreversible functional and structural changes in the body are actually delayed diabetic complications that results from ill management and poor achievement of control by the existing antidiabetic measures/ treatment. It is therefore not absurd to conclude that the available anti diabetic measures such as oral hypoglycemic agents and insulin, have not effectively controlled the delayed diabetic complications. Apart from this, these available treatment agents (oral hypoglycemic agents and insulin) possess side effects that have brought about a growing interest among group of patients finding succour in use of products having anti-diabetic activity of better performance with less toxicity and cost [5-7].

Researchers worldwide are encouraged to intensify efforts in the search for more effective treatment remedies/ agents [7-11] not withstanding that there are already available hypoglycaemic agents. Pharmacological research underpin by molecular understanding seems to have gained ground as driver for advances in controlling type 2 diabetes mellitus [12]. Many scientists are joining this quest for the best treatment agent (yet undisclosed) so as to control the frequent disease corollaries. To ease the horizon the disease simulation has to be easily achievable and possible to access by all, in the research hub.

Experimental Models

The experimental model of a disease aids not only the understanding of the pathophysiology of the disease but also the development of drugs for its treatment. There already exist various models of the disease state, from genetically engineered to chemically induced rodents, not mentioning large animals thereof and surgical options (partial and the total pancreatectomy). The ease of handling rodents makes their use most appropriate for research purposes [13], coupled with the possible deductions owing to their close relationship in physiology to humans [14,15] not leaving out the economic advantages [14] especially the resources to maintain and manage the research due to their relative availability, low cost of purchase and care. Though there exist several models of the disease in rodents, affordability, availability, and ease with use of some of these models are far reaching, limiting the options left to the low income researchers in developing countries to which most, if not all of the African institutions are- an ugly truth caused by misdirected policies of her governments. It suffices to reiterate some of these diabetes rodent models.

Genetically Engineered Diabetic Rodent Models

Spontaneously Diabetic Rodents are genetically engineered achieving the disease and subsequently bred for that purpose. There are now several genetically engineered diabetic rodent models exhibiting defects simulating the disease state. The Bio Breeding BB rat is a model of spontaneous diabetes associated with insulin deficiency and insulitis due to autoimmune destruction of pancreatic beta cells [16]. The clinical presentation of diabetes is sudden occurring about 60–120 days of age in these laboratory animals so that within several days, diabetic animals are severely hyperglycemic, hypoinsulinemic, and ketotic unless insulin treatment is instituted [16]. Another spontaneous diabetes strain is the inbred Wistar strain, WBN/Kob rat that exhibit impaired glucose tolerance and glucosuria at 21 weeks of age develop demyelinating, predominantly motor neuropathy, later accompanied by axonal changes. Yet another genetically engineered is the Cohen Diabetic Rat that presents with diabetes and diabetes related complications when on a diet rich in sucrose or refined sugars but poor in copper. But escape presentation when fed a starch or stock diet. There is the non-obese Goto–Kakizaki (GK) inbred strain of Wistar rats that spontaneously developed type II diabetes as early as 2 to 4 weeks after birth. Also there is Zucker-fatty obese rat that manifest mild glucose intolerance at an early age but however, their blood sugar level is usually normal throughout life. And there is obese Zucker Diabetic Fatty (Zdf/Drt-Fa) rat originally derived from the Zucker- fatty rat that only the males become spontaneously diabetic at 10 weeks of age with hyperglycemia of about 20mmol/l while the females only develop diabetes on being fed a high-fat diet. The Wistar fatty rat (WDF/Ta-fa rat) established hyperglycaemia by the transfer of fatty (fa) gene from the Zucker rat to Wistar Kyoto rat, though hyperglycemia is not observed in females

except by addition of sucrose to their diet. The OLETF Rats, which are maintained at the Tokushima Research Institute Japan, is also a strain of spontaneously diabetic rat occurring via by selective breeding after 18 weeks of age. Also only males express the diabetic state which could be completely prevented by administration of diazoxide (0.2% in diet) from the age of 4 to 12 weeks. Yet another spontaneously diabetic rat is the ESS-Rat with abnormal glucose tolerance tests from the age of 2 months onwards. There is the Obese SHR Rat with developed hyperglycemia and glucosuria associated with giant hyperplasia of pancreatic islets, from which several substrains were developed, like the JCR: LA-corpulent rat with impaired glucose tolerance. There is the congenic SHR/N-cp rat strain, at the National Institutes of Health, USA, exhibiting obesity, mild hypertension, hyperinsulinemia, and glucose intolerance. A BHE rat model exist in which the diabetic state manifest only at maturity of 50 days of age. Finally there is the LEW.1AR1/Ztm-iddm RAT, a Type I (insulin-dependent) diabetes mellitus rat model with diabetes appearing in them with an incidence of 20% without major sex preference at 58+/-2 days[16].

Just as for rats the Spontaneously Diabetic Mice models abound. There is a diabetic strain of the KK-mouse which at the age of seven months or older show glucosuria and blood sugar levels up to 320 mg%. Also there is the KK-Ay Mouse also know as yellow KK mice, it develop marked adiposity and diabetic symptoms from 5 weeks, to its completeness at 16 weeks of age. They can be used to demonstrate the extrapancreatic action of antidiabetic drugs. There is the NOD mouse strain, which Like the BB rat is a model of insulin dependent diabetes mellitus manifesting insulin depletion between 3 and 7 months of age though preventable by an immunmodulating drug or a soluble interleukin-1 receptor. There are also the obese hyperglycemic mice that are glycosuric, with non-fasting blood sugar levels of about 300mg%. The diabetic condition of this strain and other obese hyperglycemic mice is different from that of humans clearly. The Diabetic db/db Mice is another strain with early onset of hyperinsulinemia, hyperglycemia up to 20 to 25 mmol/l and significant nephropathy. Spontaneous maturity onset diabetes occurs in a small percentage (10–20%) of male CBA/Ca mice with inbreeding increasing the occurrence to 80% expressing at 12–16 weeks of age. The Wellesley mouse has elevated levels of immunoreactive insulin in serum, enlarged pancreatic islets. The Chinese hamster (Cricetulus griseus) has elevation of blood sugar from normal 110mg % to 600mg% with severe polyuria, glucosuria, ketonuria, and proteinuria. Apart from these spontaneous diabetic rodents there exist other Species with Inherited Diabetic Symptoms such as; the sand rat (Psammomys obesus) that develop diabetic symptoms when fed Purina laboratory chow instead of an all-vegetable diet within 2–3months; the spiny mouse (Acomys cahirinus) in which diabetes occurs in about 15% of the animals under laboratory conditions accompanied by hyperplasia of the endocrine pancreas; South African hamsters (Mystromys albicaudatus) with spontaneous diabetes mellitus; and Tuco-tucos (Ctenomis talarum) [16].

Chemical Induced Diabetic Models

It is worth noting that the period of wait, difference of onset of presentation, and individual peculiarities are the short falls limiting the use of these genetically engineered rodents apart from accessibility and affordability. The use of diabetogenic substances such as chemicals abounds [17] and has gained much use especially with rodents as the animal of choice due to certain benefits. Inclusive of these chemicals in use from searches gotten from this present review for chemical induction of diabetics are streptozotocin (STZ) in single use, alloxan(ALX) in single use, STZ- nicotinamide combined use, STZ+ high-fat diet(HFD), aflatoxin, TB antigen, Diet induced obese state and Ferric nitrilotriacetate methods.

Their use is known to cause selective loss of pancreatic β-cells that secrete insulin while maintaining intact α- and δ-cells [16]. These models have residual insulin secretion allowing the rodents live relatively long without insulin therapy. The presentation of ketosis and mortality is relatively low. And their relative ease of maintenance and cheaper purchase deal is indulging. The drawbacks though exist; inability to simulate insulin resistance and non-engagement in long term study because of instability of the state due to regeneration of pancreatic β-cell.

Streptozotocin-Induced Diabetes: The compound STZ was discovered in 1959, a broad spectrum antibiotic isolated from a soil microorganism Streptomyces acromogenes. STZ is transported into B-cells via the glucose transporter GLUT2 and causes DNA damage. The severity and onset of symptoms depend on the dose of streptozotocin employed; preferably, male Wistar rats (150–220g) are injected 60mg/kg streptozotocin intravenously. Symptoms occur after 24–48hrs with hyperglycemia, glucosuria and ketonemia but the steady state for pharmacological test is reached from 10-14 days. Various species are susceptible to streptozotocin; a single intraperitoneal injection of 50mg/kg streptozotocin has been employed on the golden Syrian hamster, and mice [16]. Additionally, in rats, 80mg/kg, administered intraperitoneally may be used [17]. From these literatures it is obvious there abound numerous dose regimen from different authors, making the use of the chemical confusing to first time researchers and at such debilitating against the progress in the research area.

Alloxan-Induced Diabetes: there is the possibility of producing different grades or severity of the disease by dose variation of alloxan given [17]. Subcutaneous injection of100–175 mg/kg alloxan to Wistar or Sprague-Dawley strain rats weighing 150-200g will ensure diabetic symptoms [16].

Different routes include intravenous dose of alloxan in rats at 65mg/kg; and if administered intraperitoneally the effective dose is 150mg/kg body following 18 hours fast. Again, with this chemical there exist ranges of doses and route employed by various researchers.

Ferric Nitrilotriacetate: is known to cause diabetes by iron loading to pancreatic endocrine cells. Rats parenterally treated with large daily dose of ferric nitrilotriacetate manifested diabetic symptoms though this method is rarely employed [18].

Streptozotocin (STZ)-Nicotinamide: Treatment with both streptozotocin and nicotinamide has been used to induce experimental diabetes in rodents. While STZ cause the pancreatic B-cell damage, the nicotinamide protect insulin secreting cells against STZ by inhibiting the enzyme polymerase activity thereby reducing the effect of necrosis on the insulin secreting cells [19].

Streptozotocin (STZ)-High-Fat Diet (HFD): The reason perceived for the method is that the HFD is expected to cause insulin resistance and coupled with the action of low dose STZ, simulate the model of type 2 diabetes state needed by most researchers [20].

Diet Induced Obese State: Components of the disease state in need to be studied may be simulated without out rightly having to make the animal diabetic. It is widely accepted to study the mechanisms that contribute to obesity-induced cardiac injury and dysfunction without hyperglycaemic component [21].

Advantages and Limitations of Chemical Induction Methods

There exist numerous advantages in the use chemical induction of diabetes. Its suitability for usage when testing medications or treatments is a ready example of its quick use [22] being that the critical purpose of most quests is dropping the blood sugar. The affordability of the chemical is another advantage though this depends on the choice of the chemical intended. The ease of avoiding human experimentation can also be taken into consideration as chemical induction of diabetes beneficial use to researches. Another benefit is the possibility of being able to harvest the pancreas for histological analysis [23] being that these experimentation is in animals. Another benefit is the choice of pathology intended for simulation (type 1 or type 2), being achievable depending on choice of dosage of the chemical used [17].

The chemically induced diabetic model's main disadvantage or limitation is the toxic effect of the chemicals on different organs [22]. True to this, the yield on inducing the animals is usually a troubling task before any study is complete, being that on trying to make say about five animals diabetic, up to 8- 10 animals would have been injected. This may include losses causing wastage [24]. A relative limitation is the difficulty posed by most international companies dealing on the chemical enforcing the purchase of these chemical being institutional based (this was faced by our team of researches). Least to mention is the danger of the chemical to researchers on mishandling which can be overcome by best practices in the laboratory.

Applications in Research

Depending on the research purpose or type of diabetes pathology researchers are pursuing, the various chemical used and dosage makes chemical induction a dependable tool in this hob. This is made obvious by the large traffic of researches using these methods as depicted in bar charts showing chemical induction of diabetes spanning the years distributed under this study (seen in the result section: research literature spanning the period). The various application range from, adaptation to analyze therapies of transplantation where the ultimate goal is to lower the blood glucose [22], to needed existence of endogenous beta cells that may be required in insulin-positive animal models [25]. It suffices to mention that male rodents are often employed due to their susceptibility to the diabetogenic action of the chemicals [24]

Comparison with Alternative Methods

As mentioned earlier the short falls limiting the use of genetically engineered rodents informed the use of the chemical models of diabetes. But it should be said that both models have their place in the research into the various pathological simulation of diabetes mellitus areas of interest vas visa its complications. There may be the surgical removal of part of the pancreas (pancreatectomy) as another model practiced [17]. It suffices to say that apart from the chemicals earlier mentioned in use for induction of diabetes, the following has been employed; dithizone(zinc-chelating agent), gold thioglucose, hormones (growth hormone and corticosteroid) and virus Induced Diabetes [26]. However, most used genetically engineered models deviate from human type 2 diabetes mellitus, being that, such posses' mutations which are not common in human type 2 diabetes [27]. Also, Islet cell transplantation studies have been utilized in rodents [xxvii]. In comparison of chemical models to other models, majority of the studies over the period reviewed is the chemical-induced diabetes models [27].

Review method

The cumbersome effort in trying to decide on the method and chemical of choice in traversing this research area gave us the impetus to delve into the research notwithstanding the richness of literature in this hub. These different routes and doses employed by researchers informed this research work meant to decipher with ease the best route and chemical to

be employed after a wide review of researches carried out spanning from 1975 to 2023. The criteria to be used for making the decision of choice are

The frequency of use of the chemical;
The most frequently used dose and route of administration;
Ease of the administration in terms of technique.

In the review the following search engine would be employed: PubMed; Google scholar; Base; World cat search and Science direct. And a table was made from which the above parameters for decision were extracted. The table contained: the chemical used, rodent employed, route of drug administration, dose employed, purpose or title of the research and the references.

Results

Research Literature Spanning the Period

An enormous amount of research work in the research area is evident, as seen from the
Bar charts showing chemical induction of diabetes spanning the years distributed

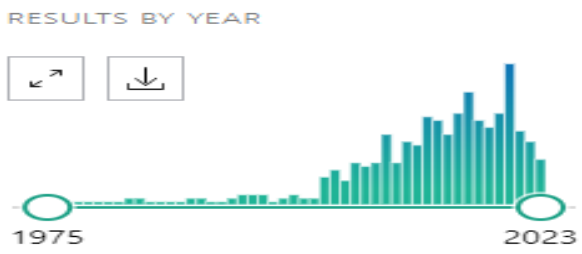

Figure 1: PubMed distribution of chemical induction 1975 – 2023.

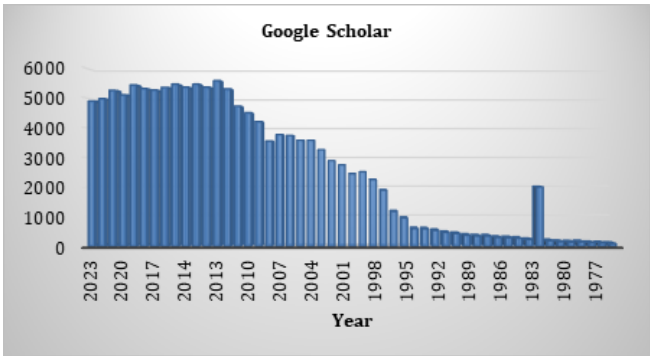

Figure 2: Google scholar distribution of chemical induction1975 - 2023.

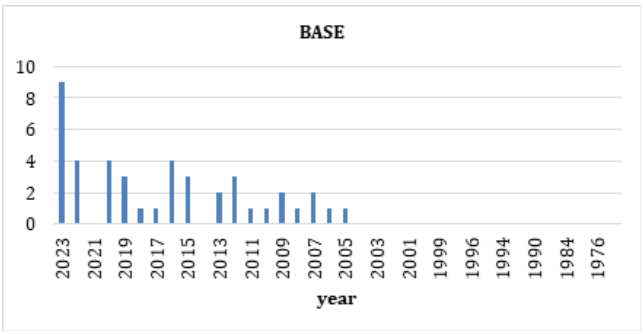

Figure 3: BASE distribution of chemical induction 1975 - 2023.

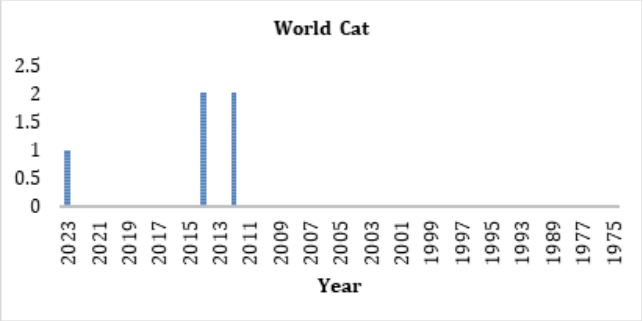

Figure 4: World cat distribution of chemical induction 1975 - 2023.

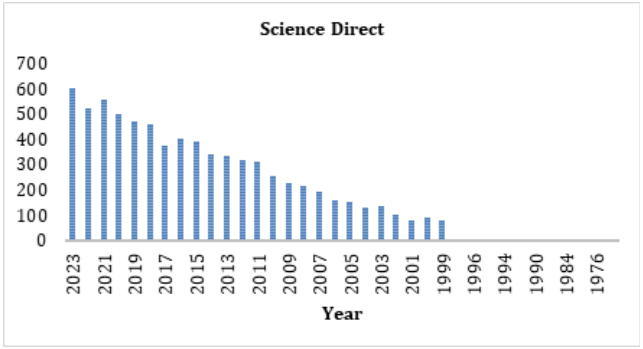

Figure 5: Science direct distribution of chemical induction 1975 - 2023.

Frequency of Chemical Used

The most frequently used chemical, using PubMed, as an example to buttress this finding (Table 1) is STZ 219 times followed by ALX 13 times. The same picture was painted in google scholar, BASE; World cat search and Science direct with STZ disproportionately more frequently used than ALX.

Because of the enormous data generated from google scholar search, the review for this search engine was limited to the first twenty most relevant literatures for each year reviewed.

Type of Chemical Method of Diabetics Induction Employed											
		STZ alone	ALX alone	Ferric Nitr-ilotriacetate	STZ-Nicot-inamide	TB antigen	Aflatoxin	Abscisic Acid	STZ+ High-Fat Diet	Diet Induced Obese	Others/ Unrelated/ Glucose Injection
Year	Count										
2023	12	7	-		1		1	1	1	1	
2022	17	6				1			1		9
2021	20	9	1						1		9
2020	39	23			1				1	1	12
2019	25	15							2		8
2018	21	8							5	1	8
2017	23	12			1				3	1	6
2016	31	17			1				1		12
2015	25	10			2				1		12
2014	19	9	1		1				1		8
2013	23	15									8
2012	24	9	2								13
2011	16	11	2								3
2010	17	11									6
2009	11	7									4
2008	19	6									13
2007	11	5	2								5
2006	10	8	2								
2005	11	6	1								4
2004	6	4	2								1
2003	9	8									1
2002	7	2									5
2001	1										1
2000	1	1									
1999	2	2									
1998	1	1									
1996	2	2									
1995	2										2
1994	2	1									1
1993	1										1
1990	1	1									
1989	1	1									
1984	1										1
1983	1	1									
1976	1	1									
1975	1										1

Table 1: shows the year per chemical count using the various chemicals possible.

Route of drug administration

The frequency of the route was noticed to be different over time. As in the earlier works, the 1975, up to the 1980s, the Intravenous route was most often employed (Table 2) while in the most recent timesup to 2023; the intraperitoneal route was most favoured (Table 3).

S/N	Chem.	Rodent Type	Purpose of Study	Route of Adm	Dose	References
1	Alloxan	Rat	Potentiation of the Hepatotoxic Responses to Chemicals in Alloxan-Diabetic Rats'# (38924)	IV	Single 60mg/kg	Hanasono GK, et al. [28]
2	Alloxan	Rat	Emergence of overt diabetes in offspring of rats with induced latent diabetes	IP	150 mg/kg	Spergel G, et al. [29]
3	Streptozotocin	Rat	Histochemical properties of skeletal muscle fibers in streptozotocin-diabetic rats	IV	single 60 mg/kg	Armstrong RB, et al. [30]
4	Streptozotocin	Rat	Studies of the Rate of Regression of the Glomerular Lesions in Diabetic Rats Treated with Pancreatic Islet Transplantation	IP	injection of 65 mg/kg of streptozotocin, intravenously, and 2 ml of a 30% glucose and water solution, intraperitoneally.	Mauer SM, et al. [31]
5	Streptozotocin	Rat	Testicular Lesions of Streptozotocin Diabetic Rats	IV	Single 75 mg/kg body weight	Oksanen A, et al. [32]
6	Alloxan	Rat	Isoproterenol- And Epinephrine-Induced Changes In Blood Glucose And Tissue Glycogen Levels In Normal And Diabetic Rats: The Influence Of Alteration In Endogenous Insulin Levels And State Of Nourishment	IV	Single 50 mg/kg	Potter DE, et al. [33]
7	Streptozotocin	Rat	Islet Transplantation in Experimental Diabetes of the Rat - I. Comparative Studies: Pancreatectomy – Streptozotocin	IV	80, 100 or 120 mg/kg, Others 65 mg/kg; 150 mg/kg.	Slijepcevic, M, et al. [34]
8	Streptozotocin	Rat	Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes.	IV	70mg/kg	Greene DA, et al. [35]
9	Alloxan	Rat	Potentiation of carbon tetrachloride-induced	IV	40 or 80 mg/kg	Hanasono GK, et al. [36]
	Streptozotocin	Rat	Hepatotoxicity in alloxan- or streptozotocin-diabetic rats	IV	65mg/kg	

10	Alloxan	Rat	Studies on the effects of alloxan and streptozotocin induced diabetes on lipid metabolism in the isolated perfused rat lung	IV	45mg/kg	Moxley MA, et al. [37]
	Streptozotocin	Rat		IV	65mg/kg	
11	Streptozotocin	Rat	Cell Membrane Changes in Chronically Diabetic Rats	IV	65mg/kg	Chandramouli V, et al. [38]
12	Alloxan	Rat	The regulation of phosphoenolpyruvate carboxykinase (GTP) synthesis in rat kidney cortex. The role of acid-base balance and glucocorticoids	SC	90mg/kg	Iynedjian PB, et al. [39]
13	Alloxan	Mice	Effects of alloxan diabetes, anti-insulin serum diabetes, and non-diabetic dehydration on brain carbohydrate and energy metabolism in young mice.	IV	100mg/kg	Thurston JH, et al. [40]
14	Streptozotocin	Rat	Dichloroacetate-lnduced Changes in Liver of Normal and Diabetic Rats.	IV	50mg/kg	Anderson, JW, et al. [41]
15	Streptozotocin	Rat	Glycogen synthesis in the perfused liver of streptozotocin-diabetic rats	IV	75mg/kg	Whitton, PD, et al. [42]
16	Alloxan	Rat	Metabolic and histopathologic changes in arteriosclerotic versus nonarteriosclerotic rats following isoproterenol-induced myocardial infarction with superimposed diabetes	SC	10 mg /100 g, b.w.	Wexler BC [43]
17	Alloxan	Rat	Insulin and glucose as modulators of the amino acid-induced glucagon release in the isolated pancreas of alloxan and streptozotocin diabetic rats.	IV	40mg/kg	Pagliara AS, et al. [44]
	Streptozotocin	Rat		IV	65-70mg/kg	
18	Alloxan	Rat	Role of carnitine in hepatic ketogenesis	IV	70mg/kg	McGarry JD, et al. [45]
19	Alloxan	Rat	The effects of fasting, diabetes, and hypophysectomy on adipose lactic dehydrogenase isozymes.	SC	20mg/ 100g	Hern EP, et al. [46]
20	Streptozotocin	Rat	Islet Transplantation in Experimental Diabetes in the Rat	IV	65, 80, 100, 120 and 150 mg/kg bw	Slijepcevic, M, et al. [47]

Table 2: table showing route of drug administration, chemical & rodent use in 1975 google scholar search employing the first twenty most relevant literatures.

S/N & Chemical Employed	Rodent Type	Purpose of Study	Route of Administration	Dose	References
1st STZ	Mouse	CCR2-positive monocytes contribute to the pathogenesis of early diabetic retinopathy in mice	ip	60mg/kg for	Saadane A, et al. [48]
2nd STZ	Mice	Metformin ameliorates ROS-p53-collagen axis of fibrosis and dyslipidemia in type 2 diabetes mellitus-induced left ventricular injury	ip	50mg/kg	Al-Ani B, et al. [49]
3rd ALX	Rat	Antidiabetic activity of mango peel extract and mangiferin in alloxan-induced diabetic rats.	IP	150mg/kg	Mistry J, et al. [50]
4th STZ	Rat	Protective effect of quercetin on pulmonary dysfunction in streptozotocin-induced diabetic rats via inhibition of NLRP3 signaling pathway	IP	50Mg/kg	El-Shaer NO, et al. [51]
5th STZ	Rat	Dose-dependent effects of taurine against testicular damage in a streptozotocin-induced type 1 diabetes mellitus rat model	ip	55mg/kg	ElBanna AH, et al. [52]
6th STZ	Rat	Ezetimibe attenuates experimental diabetes and renal pathologies via targeting the advanced glycation, oxidative stress and AGE-RAGE signalling in rats.	ip	60mg/kg	Nabi R, et al. [53]
7th STZ	Rats	Assessment of renal function in diabetic Wister rats treated with ethanol extract of Cucumis sativus FRUIT.	ip	50 mg/kg	Abu O, et al. (2023) [54]
8th STZ	Mouse	Cardiovascular characterisation of a novel mouse model that combines hypertension and diabetes co-morbidities.	ip	55mg/kg daily for 5 days	Sharma A, et al. [55]
9th STZ	Rat	Attenuation of Oxidative Stress and Nephrotoxicity with Supplementation of Pimpinella tirupatiensis Tuberous Root in Streptozotocin-induced Diabetic Rats	iv	40mg/kg	Lavanya T, et al. [56]
10th STZ	Rat	Renal Oxidative Status in Diabetic Wistar Rats Administered Methanol Fraction of Ethanol Extract of Dialium guineense Stem Bark.	ip	50mg/kg	Alegun DOA, et al. [57]

11th STZ	Rat	Effects of 4-phenylbutyric acid on the development of diabetic retinopathy in diabetic rats: regulation of endoplasmic reticulum stress-oxidative activation	ip	35mg/kg	Abdel-Ghaffar A, et al. [58]
12th ALX	Rat	Hypoglycemic effect of betulin in rats with experimental diabetes	ip	170mg/kg	Zakrzeska A, et al. [59]
13th STZ	Rat	Dalbergiella welwitschia (Baker) Baker f. alkaloid-rich extracts attenuate liver damage in streptozotocin-induced diabetic rats.	Ip	45mg/kg	Ajiboye BO, et al. [60]
14th Nicotinamide and STZ	Rat	Safety of Tithonia diversifolia (Hemsley) a gray ethanol extracts in diabetic-induced rats.	Ip	nicotinamide 230 mg/kg and streptozotocin 65 mg/kg	Istikharah R, et al. [61]
15th Nicotinamide and STZ	Rat	Metabolic Alterations in Streptozotocin–nicotinamide-induced Diabetic Rats Treated with Muntingia calabura Extract via 1H-NMR-based Metabolomics.	Ip	nicotinamide mg/kg and streptozotocin mg/kg	Zolkeflee NKZ, et al. [62]
16th STZ +HFD	Rat	Chitosan-encapsulated nano-selenium targeting TCF7L2, PPARγ, and CAPN10 genes in diabetic rats	Ip	Stz 50mg/kg	Abozaid OA, et al. [63]
17th ALX	Rat	Evaluation of blood glucose, hepatic biomarkers and histopathology of alloxan induced diabetic rats fed Sesamum indicum compounded diet	Ip	120mg/kg	Ekeke KL, et al. [64]
18th ALX	Rat	Instability of alloxan-induced diabetes and its impact on sex and thyroid hormones in male wistar rats-a pilot study.	Ip	150mg/kg	Osibemhe M, et al. [65]
19th ALX	Rat	Hypoglycemic Effect of Olive Oil (Olea europea) on Alloxan-Induced Diabetic Albino Rats.	Ip	150mg/kg	Zakari A, et al. (2023) [66]

Table 3: table showing route of drug administration, chemical & rodent use in 2023 google scholar search employing the first twenty most relevant literatures.

Dose Employed

STZ: The route and rodent employed tend to determine the dose used as well as the chemical employed. In rats, the common doses employed for IP injection of STZ alone range from 35 mg/kg to 70mg/kgwith the range of 35- 65mg /kg most often used.

ALX: Dose ranged between 50 -150 by IV/IP injection were most often used.

Route Employed

A large number of researchers lean on intraperitoneal (IP) route over intravenous (IV) route with the distribution showing the earlier times of the 2000s having more of IP and the 1975 up to 1980s using more of IV.

Rodent Used

Rats were most often used than mice; male rats were most often preferred.

Discussion

Reviewers in this hub at different times have commented on the most commonly or frequently used chemical models. In this research, the most frequently engaged methodsare the two chemical methods, streptozotocin and alloxan respectively, which is in keeping with other researchers, Lukacínová, 2013[3]. It suffices to know of the two chemical, alloxan was first found to induce diabetes since 1943 [67] while STZ was later discovered in 1959 [68]. The choice of STZ over ALX might be due to the relative ease of achieving diabetic state using low dose with relative stability of the rodents(on administration of 10% sucrose solution) allowing a larger amount of animal to be engaged in any chosen research work [69].

The review saw doses of STZ mostly below 70mg/kg being applied or engaged (ie between 35- 65mg/kg). This is believe to be due to the fact that the direct effect of STZ is associated with dose, reflecting an increase mortality at very high doses especially beyond 70mg/kg body weight [30] causing a low yield of animals to be engaged in the final research work. This dose dependant mortality effect can be reduced on application of Tacrine, a glycation scavenger, administered after STZ administration to protect the rodents from the lethality of STZ, thereby reducing mortality [70]. Also giving of sugars like D-glucose and D-mannose before and after STZ injection can protect the animals from the STZ mortality effect. This advantage is not seen with ALX as no effort as above, can reduce mortality following its administration. The lethality in the case of STZ's is its toxicity on multiple organs [24]. Also the dose of ALX often engaged is in the range between 70- 170mg/kg.

The route of administration of both STZ and ALX saw a tilt towards intraperitoneal injection in the present times (2000s up to 2023) than the earlier time (1970s, 1980s). This preference may have been informed by the less precision needed in technique or proficiency of the researcher being that the IV route might be more difficult to execute, as even locating the veins is quite cumbersome. This argument was put forte because literature supports the advantage of IV administration because accidental delivery of the drug into the bowel or sub peritoneal space might increase mortality and reduce efficacy [30] using IP, which is avoidable IV. Additionally, the IP route is commonly used in rodents because it can be used for the delivery of larger volumes than an intravenous (IV), intramuscular (IM) or subcutaneous route.

The preferred rodent in most research work was rats and the male sex over the female. This is because the diabetogenic effect of the drug is interfered with by oestrogen of the female gender resulting in need for higher doses [30].

Conclusion

The most frequently engaged chemical methods still remains the two chemicals, streptozotocin and alloxan induction respectively. The preferred route and dose for streptozotocin for chemical induction are Intraperitoneal with the range of between 35- 65mg/kg; and that of Alloxan also were Intraperitoneal and dose range of between 70-170mg/kg. The preferred rodents in most research work were rats and the male sex over the female.

In furtherance to this review, the investigators intend to use the recommended chemical, using the parameters; route (IP), doses (range), rodent (male rat) to confirm their findings taking precautions recommended within. Conclusively, it is important to state the obvious drawbacks of genetically engineered and or the laboratory animals with chemically induced diabetics model includes; the lack of full evolutionary processes of the human disease complexities and the lack of representation of the progressive nature of human diabetics respectively.

Declarations

Ethics approval and consent to participate
Members of this research team collectively won a grant for which this review is part of, and have given their consent to participate.
Ethics approval - not applicable.

Consent for Publication

The members have also given their consent for the current publication
Availability of data and materials
There is available data for this publication being embarked on.

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References

1. World Health Organization (1999) Diabetes mellitus. Facts sheet numbers 138 and 236, Geneva.
2. World Health Organization (2023) Diabetes mellitus. Facts sheet 5 April, 2023
3. Lukacinova A, Hubkova B, Rácz O, Ništiar F (2013) Animal Models for Study of Diabetes Mellitus (chapter 13): In Diabetes Mellitus–Insights and Perspectives. IntechOpen.
4. Edwards CRW, Band JD, Frier BM, Shephend JADT (1995) In: Davisons principles and practices of medicine (17th). Endocine and metabolic diseases, including diabetes mellitus (chapter 12). ELBS low priced books.
5. Holman RR, Turner RC (1991) Oral Agents and Insulin in the Treatment of NIDDM. In: Pickup J, Williams G (Eds.), Text Book of Diabetes, Blackwell, Oxford, pp: 467-469.
6. Wanderley JDM, Marcos OM, Manoel EF, Gabriel MPDM, Valéria PDM (2002) Chemical properties and enzyme activity in a sewage sludge-treated soil, Communications in Soil Science and Plant Analysis 33(9-10): 1643-1659.
7. World Health Organization (2013) 10 Facts about diabetes. Fact file.
8. World Health Organization (1994). WHO study Group report on prevention of diabetes mellitus [WHO technical report series No 844]. WHO Geneva, pp: 181.1
9. Keshawarz A, Pyle L, Alman A, Sassano C, Westfeldt E, et al. (2019) Type 1 Diabetes Accelerates Progression of Coronary Artery Calcium Over the Menopausal Transition: The CACTI Study. Diabetes Care 42(12): 2315-2321.
10. (2020) NIHR Programme Grants for Applied Research (PGfAR) and Diabetes UK PGfAR.
11. Hopkins M, Andrews R, Salem V, Taylor R, Le RC, et al. (2020) Improving understanding of type 2 diabetes remission: research recommendations from Diabetes UK's 2019 remission workshop. Diabet Med 37(11): 1944-1950.
12. Shomali M (2025) Diabetes treatment in 2025: can scientific advances keep pace with prevalence? Ther Adv Endocrinol Metab 3(5): 163-173.
13. (2004) Thomas Buerge and Tilla Weiss Handling and Restraint in The Laboratory Mouse (Handbook of Experimental Animals) by T Buerge.
14. Bryda EC (2013) The Mighty Mouse: the impact of rodents on advances in biomedical research. Mo Med 110(3): 207-211.
15. Michael VM, Andrei S (2016) In Handbook of the Biology of Aging 8th (Edn.).
16. Gerhard V (2008) In Drug Discovery and Evaluation: Pharmacological Assays. Third Completely Revised, Updated and Enlarged Edition.
17. Etuk EU (2010) Animals models for studying diabetes mellitus. Agriculture And Biology Journal Of North America.
18. Awai M, Narasaki M, Yamanoi Y, Seno S (1979) Induction of diabetes in animals by parenteral administration of ferric nitrilotriacetate. A model of experimental hemochromatosis. Am J Pathol 95(3): 663-673.
19. Szkudelski T (2012) Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Experimental Biology and Medicine 237(5): 481-490.
20. Magalhaes DA, Kume WT, Correia FS, Queiroz TS, Allebrandt NEW, et al. (2019) High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: a new proposal. An Acad Bras Cienc 91(1): e20180314.
21. Sciarretta S, Zhai P, Shao D, Maejima Y, Robbins J, et al. (2012) Rheb Is a Critical Regulator of Autophagy during Myocardial Ischemia. Circulation 125(9): 1134-1146.
22. Sasmita D, Gurudutta P, Biswakanth K, Nityananda S, Sanjib B (2021) An Approach Towards Method Development to Investigate the Anti-Diabetic Activity on Experimental Animals. Current Trends in Biotechnology and Pharmacy 15(3): 330-348.
23. King AJ (2012) The use of animal models in diabetes research. Br J Pharmacol 166(3): 877-894.
24. Goyal SN, Reddy NM, Patil KR, Nakhate KT, Ojha S, et al. (2016) Challenges and issues with streptozotocin-induced diabetes-A clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics. Chemico-Biological Interactions 244(2016): 49-63.
25. Kargar C, Ktorza A (2008) Anatomical versus functional beta-cell mass in experimental diabetes. Diabetes Obes Metab 10(Suppl. 4): 43-53.

26. Vineeta T, Janeshwer V (2014) Different models used to induce diabetes: a comprehensive review. International Journal of Pharmacy and Pharmaceutical Sciences 6(6).

27. Duncan MM, Morris K, Faraj O, Alkizim (2012) Peculiar glycemic patterns in alloxaninduced diabetes animal model. Afr J Pharmacol Ther 1(1): 30-34.

28. Hanasono GK, Witschi H, Plaa GL (1975) Potentiation of the Hepatotoxic Responses to Chemicals in Alloxan-Diabetic Rats. Proceedings of the Society for Experimental Biology and Medicine 149(4): 903-907.

29. Spergel G, Khan F, Goldner MG (1975) Emergence of overt diabetes in offspring of rats with induced latent diabetes. Metabolism 24(12): 1311-1319.

30. Armstrong RB, Gollnick PD, Ianuzzo CD (1975) Histochemical properties of skeletal muscle fibers in streptozotocin-diabetic rats. Cell Tissue Res 162(3): 387-393.

31. Mauer SM, Steffes MW, Sutherland DER, Najarian JS, Michael AF, et al. (1975) Studies of the Rate of Regression of the Glomerular Lesions in Diabetic Rats Treated with Pancreatic Islet Transplantation. Diabetes 24(3): 280-285.

32. Oksanen A (1975) Testicular Lesions of Streptozotocin Diabetic Rats. Hormone Research 6(3):138-144.

33. Potter DE, Ellis S (1975) Isoproterenol- And Epinephrine-Induced Changes In Blood Glucose And Tissue Glycogen Levels In Normal And Diabetic Rats: The Influence Of Alteration In Endogenous Insulin Levels And State Of Nourishment. J Pharmacol Exp Ther 193(2): 576-84.

34. Slijepcevic M, Helmke K, Federlin K (1975) Islet Transplantation in Experimental Diabetes of the Rat - I. Comparative Studies: Pancreatectomy - Streptozotocin. Hormone and Metabolic Research 7(1): 20-25.

35. Greene DA, Jesus Jr PVD, Winegrad AI (1975) Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes. J Clin Invest 55(6): 1326-1336.

36. Hanasono GK, Cote MG, Plaa GL (1975) Potentiation of carbon tetrachloride-induced Hepatotoxicity in alloxan- or streptozotocin-diabetic rats. J Pharmacol Exp Ther 192(3): 592-604.

37. Moxley MA, Longmore WJ (1975) Studies on the effects of alloxan and streptozotocin induced diabetes on lipid metabolism in the isolated perfused rat lung. Life Sciences 17(6): 921-925.

38. Chandramouli V, Carter JR (1975) Cell Membrane Changes in Chronically Diabetic Rats. Diabetes 24(3): 257-262.

39. Iynedjian PB, Ballard FJ, Hanson RW (1975) The regulation of phosphoenolpyruvate carboxykinase (GTP) synthesis in rat kidney cortex. The role of acid-base balance and glucocorticoids. Journal of Biological Chemistry 250(14): 5596-5603.

40. Thurston JH, Hauhart RE, Jones EM, Ater JL (1975) Effects of alloxan diabetes, anti-insulin serum diabetes, and non-diabetic dehydration on brain carbohydrate and energy metabolism in young mice. Journal of Biological Chemistry 250(5): 1751-1758.

41. Anderson JW, Karounos D, Yoneyama T (1975) Dichloroacetate-lnduced Changes in Liver of Normal and Diabetic Rats. Experimental Biology and Medicine 149(3): 814-821.

42. Whitton PD, Hems DA (1975) Glycogen synthesis in the perfused liver of streptozotocin-diabetic rats. Biochemical Journal 150(2): 153-165.

43. Wexler BC (1975) Metabolic and histopathologic changes in arteriosclerotic versus nonarteriosclerotic rats following isoproterenol-induced myocardial infarction with superimposed diabetes. Metabolism 24(12): 1321-1337.

44. Pagliara AS, Stillings SN, Haymond MW, Hover BA, Matschinsky FM (1975) Insulin and glucose as modulators of the amino acid-induced glucagon release in the isolated pancreas of alloxan and streptozotocin diabetic rats. The Journal of Clinical Investigation 55(2): 244-255.

45. McGarry JD, Robles-Valdes C, Foster DW (1975) Role of carnitine in hepatic ketogenesis. Proceedings of the National Academy of Sciences 72(11): 4385-4388.

46. Hern EP, Schultz J, O'Dorisio MS, Moore RO, Serif GS (1975) The effects of fasting, diabetes, and hypophysectomy on adipose lactic dehydrogenase isozymes. Archives of Biochemistry and Biophysics 169(1): 331-338.

47. Slijepcěvić M, Helmke K, Federlin K (1975) Islet Transplantation in Experimental Diabetes in the Rat. Hormone and Metabolic Research 7(6): 456-461.

48. Saadane A, Veenstra AA, Minns MS, Tang J, Du Y, et al. (2023) CCR2-positive monocytes contribute to the pathogenesis of early diabetic retinopathy in mice. Diabetologia 66: 590-602.

49. Al-Ani B, Alzamil NM, Hewett PW, Al-Hashem F, Bin-Jaliah

I, et al. (2021) Metformin ameliorates ROS-p53-collagen axis of fibrosis and dyslipidemia in type 2 diabetes mellitus-induced left ventricular injury. Archives of Physiology and Biochemistry 129(3): 734-740.

50. Mistry J, Biswas M, Sarkar S, Ghosh S (2023) Antidiabetic activity of mango peel extract and mangiferin in alloxan-induced diabetic rats. Future Journal of Pharmaceutical Sciences 9: 22.

51. El-Shaer NO, Hegazy AM, Muhammad MH (2023) Protective effect of quercetin on pulmonary dysfunction in streptozotocin-induced diabetic rats via inhibition of NLRP3 signaling pathway. Environ Sci Pollut Res 30: 42390-42398.

52. ElBanna AH, Osman AS, Hossny A, ElBanna H, El-Ela FIA (2023) Dose-dependent effects of taurine against testicular damage in a streptozotocin-induced type 1 diabetes mellitus rat model. International Journal of Immunopathology and Pharmacology 37.

53. Nabi R, Alvi SS, Shah A, Chaturvedi CP, Faisal M, et al. (2021) Ezetimibe attenuates experimental diabetes and renal pathologies via targeting the advanced glycation, oxidative stress and AGE-RAGE signalling in rats. Archives of Physiology and Biochemistry 129(4): 831-846.

54. Abu O, Awhin EP, Iyare H (2023) Assessment of renal function in diabetic wistar rats treated with ethanol extract of *Cucumis sativus* fruit. African Journal of Health, Safety and Environment 4(1): 101-107.

55. Sharma A, Choi JSY, Watson AMD, Li L, Sonntag T, et al. (2023) Cardiovascular characterisation of a novel mouse model that combines hypertension and diabetes co-morbidities. Sci Rep 13: 8741.

56. Lavanya T, Mohan KC, Reddy KS, Reddy SR (2023) Attenuation of Oxidative Stress and Nephrotoxicity with Supplementation of Pimpinella tirupatiensis Tuberous Root in Streptozotocin-induced Diabetic Rats. Pharmacognosy Research15(4): 813-818.

57. Abu O, Alegun OD, Ifekwe JC (2023) Renal Oxidative Status in Diabetic Wistar Rats Administered Methanol Fraction of Ethanol Extract of Dialium guineense Stem Bark. Medi Clin Case Rep J 1(1): 12-15.

58. Abdel-Ghaffar A, Elhossary GG, Mahmoud AM, Elshazly AHM, Hassanin OA, et al. (2021) Effects of 4-phenylbutyric acid on the development of diabetic retinopathy in diabetic rats: regulation of endoplasmic reticulum stress-oxidative activation. Archives of Physiology and Biochemistry 129(4): 964-974.

59. Zakrzeska A, Kitlas P, Shlyahtun A, Krasinska N, Jablonski R, Tomulewicz M (2023) Hypoglycemic effect of betulin in rats with experimental diabetes. Acta Poloniae Pharmaceutica 80(5).

60. Ajiboye BO, Dada S, Fatoba HO, Lawal OE, Oyeniran OH, et al. (2023) Dalbergiella welwitschia (Baker) Baker f. alkaloid-rich extracts attenuate liver damage in streptozotocin-induced diabetic rats. Biomedicine & Pharmacotherapy 168: 115681.

61. Istikharah R, Frayuda EJ, Maulida R, Pramundita A, Nugrahaningsih DAA, et al. (2023, May) Safety of Tithonia diversifolia (Hemsley) a gray ethanol extracts in diabetic-induced rats. In AIP Conference Proceedings 2720(1).

62. Zolkeflee NKZ, Wong PL, Maulidiani M, Ramli NS, Azlan A, et al. (2023) Metabolic Alterations in Streptozotocin–nicotinamide-induced Diabetic Rats Treated with Muntingia calabura Extract via 1H-NMR-based Metabolomics. Planta medica, 89(9): 916-934.

63. Abozaid OA, El-Sonbaty SM, Hamam NM, Farrag MA, Kodous AS (2023) Chitosan-encapsulated nano-selenium targeting TCF7L2, PPARγ, and CAPN10 genes in diabetic rats. Biological Trace Element Research 201(1): 306-323.

64. Ekeke KL, Iheanacho KM, Nzebude CP, Anakor DO, Udebunu GO (2023) Evaluation of blood glucose, hepatic biomarkers and histopathology of alloxan induced diabetic rats fed Sesamum indicum compounded diet. World Journal of Advanced Research and Reviews 19(2): 751-761.

65. Osibemhe M, Orji B, Omaji G, Amune E, Ezekiel J (2023) Instability of alloxan-induced diabetes and its impact on sex and thyroid hormones in male wistar rats-a pilot study. Kuwait Journal of Science, 50(1A).

66. Zakari A, Umar H, Auwal I (2023) Hypoglycemic Effect of Olive Oil (*Olea europea*) on Alloxan-Induced Diabetic Albino Rats. Nigerian Journal of Chemical Research 28(1): 51-58.

67. Srinivasan K, Ramarao P (2007) Animal models in type 2 diabetes research: An overview. Indian J Med Res 125(3): 451-472.

68. Goud BJ, Dwarakanath V, Chikka swamy BK (2015) Streptozotocin - A Diabetogenic Agent in Animal Models. Ijppr Human 3(1): 253-269.

69. Ramzy MM, El-Sheikh AA, Kamel MY, Abdelwahab SA, Morsy MA (2014) Mechanism of testicular protection

of carvedilol in streptozotocin-induced diabetic rats. Indian J Pharmacol 46(2): 161-165.

70. Kang HS, Yang H, Ahn C, Kang HY, Hong EJ, et al. (2014) Effects of xenoestrogens on streptozotocin-induced diabetic mice. J Physiol Pharmaco 65(2) 273-282.