Review Article

Animal Models for the Assessment of Antidiabetic Activity

ABSTRACT

Diabetes mellitus is a chronic condition that affects a large number of people. The fundamental cause of this condition is a lack of insulin synthesis or reduced insulin action. A large variety of in vivo and in vitro models have been developed to investigate the antidiabetic effect's fundamental mechanism. Several approaches for determining the anti-diabetic properties of plant extracts or isolated chemicals have been reported to be successful. This review covers major animal models used for the study of antidiabetic effect of a drug or substance, that could help in the development of new medicine to treat diabetes more effectively and with fewer or no adverse effects.

Keywords: Diabetes, medicinal herbs, flavonoids, and animal models

INTRODUCTION

One of the key instruments for developing an efficient model to research the mechanism of action as well as to investigate the efficacy of the active principles and plants purported to have antidiabetic potentials are animal models. Furthermore, because of the disease's heterogeneous character, there are several possible causes of both diabetes and the illnesses it is associated with. Because there are several forms of diabetes mellitus, it is thus impossible to use a single animal model to study the drug's effectiveness [1-5].

In this case, normal control and diabetic control are utilized, respectively, to measure the hypoglycaemic situation in non-diabetic animals and diabetic animals with impaired glucose tolerance. TNF-alpha is further employed to develop an insulin-resistant diabetes model [6].

Due to the variations in hepatic metabolism between humans and rats, it is quite concerning that the active components of medicinal herbs may not be tested efficiently in decreasing blood sugar levels. The metabolic process involves numerous phases, thus the active ingredients must go through each step of the route before reaching the body, where they function as metabolites [7-9].

Due to differences in absorption, distribution, metabolism, and elimination (ADME) between species [2,10-12], the sensitivity of the same active principles may change. Among all the animal models, rodents have been the most frequently employed in research on diabetes for a variety of reasons, including the ease with which the disease may be induced in rats and the comparatively cheap cost of maintenance, both of which greatly reduce the cost of the experiment. Furthermore, rats are substantially more often than any other animal species to experience genetic abnormalities that cause diabetes [13–15].

Experimental models for diabetes mellitus in vivo animals

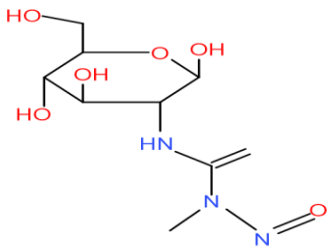
Chemicals, surgery, or genetic alterations can all be used to cause diabetes mellitus (see Table no. 2). Rodents are frequently used in diabetes research, even though certain studies have demonstrated that bigger animals may also be used to cause diabetes [16–18]. Dog pancreatectomy procedures have reportedly been performed using a big animal surgical model [19]. In another investigation, diabetes-prone strains were given to the animals with

their induces [20,21]. Depending on the method used to induce diabetes, the categories for diabetic models might be further subdivided. The following are the more specific animal models:

1. A diabetes model that was developed spontaneously, such as a CBA/Ca mouse or an ob/ob mouse.
2. A diabetic model established by experiment (e.g., surgically created by pancreatectomy; chemically induced by alloxan or streptozotocin.).

Chemicals are the cause of diabetes the term "Diabetogenic agents" refers to three categories of chemical compounds, including those that directly damage beta-cells,momentarily obstruct insulin production and/or secretion, and reduce insulin's capacity to stimulate metabolism in the target tissue.the model are outlined in the text that follows accordings to use chemicals that cause diabetes[22].

1) Streptozocin (STZ) induced diabetes:



Chemical structure of streptozocin (fig. 1)

Since 1967, glucosamine-nitrosourea drug streptozocin(STZ) has been the subject of clinical trials.the US adopted names council has reduced the name of the substance,which was earlier known as streptozocin(STZ).the upjohn co. (Kalamazoo,MI) has submitted a new drug application to the us food and drug administration to sell under the brand name Zanosar. This application will soon be approved [23]. diabetes is brought on by STZ in practically all animals.STZ can cause diabetes either with a single injection or many low-dose injections.the most used medication for causing diabetes in rats is STZ [24].Adult wistar rats are injected iv with a dosage of 60mg/kg of streptozotocin,which results in pancreatic enlargement,langerhans islet beta cell degeneration,and the development of experimental diabetes mellitus within 2-4dqys.diabetes was induced in all mice three days after beta cells started to degenerate.nicotinamide adenine dinucleotide (NAD) affects the histopathology of beta cells in the pancreatic islet and likely plays a role in the progression of diabetes [25].
Even though rats are a common animal species,other species have also been utilized. A single intravenous dose of streptozotocin (65mg/kg body weight)was used in research to induced diabetes in male new zealand rabbits. The purpose of the study was to look into the biochemical and histomorphology alterations brought on by streptozotocin induced diabetes in rabbit [26].

Streptozotocin (STZ)-treated diabetic pigs have had their insulin-mediated glucose metabolism studied to determine if they might serve as a good model for insulin-resistant type 2 diabetes mellitus. In pigs fed a low-fat diet, this study found that a steady infusion of STZ (130 mg/kg) causes the typical metabolic anomalies of type-2 diabetes mellitus and increases its susceptibility to oral metformin treatment. As a result, it makes a good humanoid animal model for researching many aspects of metabolic alterations associated with type 2 diabetes mellitus. Most likely related to hyperglycemia and/or hyperlipidaemia, insulin resistance in STZ-diabetic pigs is metabolic [27]. Streptozotocin (STZ), which is toxic to

pancreatic beta cells preferentially, is frequently used to simulate Type-1 diabetes in a variety of animals, including nonhuman primates. Vervet monkeys (Chlorocebus aethiops) were given either 45 mg/kg or 55 mg/kg of STZ intravenously to induce diabetes mellitus, whereas ten control (CTL) monkeys received saline. Exogenous insulin needs grew quickly for four weeks; following that, the insulin dose of STZ-45 steadied while the dose of STZ-55 kept rising for another 16 weeks.

Testing for glucose tolerance and insulin secretion induced by arginine revealed an 80–90% loss in pancreatic beta cell activity in both groups. All STZ monkeys had decreased body weight, and only STZ-45 had returned to baseline at 16 weeks. The STZ-55 group had elevated blood urea nitrogen and creatinine levels. In contrast to STZ-45, where alkaline phosphatase elevation was still present after the research, STZ-55 caused a rise in alkaline phosphatase (p 0.05 against control). Red cell characteristics were decreased in all STZ monkeys, but the STZ-55 group saw a greater reduction. This experiment showed that a single dosage of STZ may develop and sustain diabetic Mellitus in vervets. The toxicity profile was greatly improved by the lower dosage of STZ (45 mg/kg) without affecting the effectiveness of producing diabetes mellitus. Last but not least, it is advised to provide enough time after induction to address temporary renal, hepatic, and hematologic abnormalities [28].

By giving the musk shrew (Suncus murinus, Insectivora) a single high-dose intraperitoneal injection of STZ at a rate of 100 mg/kg body weight, researchers were able to induce severe IDDM (insulin-dependent diabetic Mellitus). According to the evidence from this model, IDDM in shrews caused by large dosages of STZ is a distinct model distinguished by fatty liver and hyperlipidemia and may help research IDDM's lipid metabolism [29]. Additionally, according to the literature, STZ can cause diabetes in bulls and cows at doses of 75 to 150 mg per kilogram of body weight. Future attempts to use STZ to cause diabetes in cattle should take into account different doses and techniques [30].

(A) Rat model of neonatal streptozotocin-induced diabetes (n-STZ):

The n-STZ model displays many phases of Type-2 diabetes mellitus, including reduced glucose tolerance and mild, moderate, and severe glycemia [31]. Giving a single dosage of STZ (100 mg/kg intravenously for a one-day-old pup and 120 mg/kg intravenously for a pup that is two, three, or five days old) causes diabetes. The cells of n-STZ rats resemble the insulin secretory features seen in Type-2 diabetes mellitus humans. As a result, the n-STZ model is one of the reliable animal models for Type-2 diabetes mellitus [32].

(B) Diabetic model induced by nicotinamide-streptozotocin (NAD-STZ):

The benefit of this paradigm is that it can induce a unique experimental diabetes condition in adult rats that resembles NIDDM more closely than other known animal models for insulin responsiveness to glucose and sulfonylureas. This model also has the advantage of partial protection from the cytotoxic impact of streptozotocin (STZ) exerted by appropriate doses of nicotinamide. Among the various dosages of nicotinamide tested in 3-month-old Wistar rats (100-350 mg/kg body wt), the dose of 230 mg/kg, given intraperitoneally, 15 min before STZ administration (65 mg/kg i.v.) yielded moderate and stable non-fasting hyperglycemia (155 ± 3 vs. 121 ± 3 mg/dl in controls; $P < 0.05$) and 40% preservation of pancreatic insulin stores in maximum animals [33]. A single intraperitoneal injection of STZ (60 mg/kg) plus NAD (120 mg/kg) in rats resulted in the development of non-insulin-dependent diabetic mMellitus(NIDDM). By scavenging free radicals, the antioxidant NAD protects cells

against the cytotoxic effects of STZ while causing very modest harm to the pancreatic beta cells that produce type 2 diabetes. Consequently, this model is viewed as a useful tool for the evaluation of insulinotropic drugs for the treatment of type-2 diabetes [34].

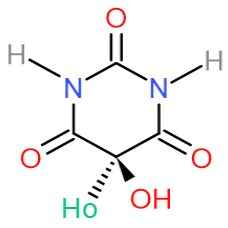
(C) Streptozotocin-induced diabetic rat model under glucose stress (STZ-S):

In this paradigm, male albino rats used in the sucrose-loaded model (SLM) were examined for in vivo antidiabetic efficacy. We utilized Charles Foster/Wistar strain rats with average body weights of 160 20 g. 60 mg/kg of STZ, which was dissolved in a 100 mM citrate buffer with a pH of 4.5, was intraperitoneally administered to overnight starved rats. Animals were classified as having diabetes if their blood glucose levels were between 144 and 270 mg/dl (8-15 mM) or higher 48 hours later when measured by glue strips. 30 minutes later, a 2.5 g/kg body weight dose of sugar was administered. Thirty minutes after the sucrose load, the blood sugar levels were once more measured using glue strips at 30, 60, 90, 120, 180, 240, and 300 min, respectively, and at 24 h. non-responders were defined as animals that were not diabetic 24 hours after the test sample had been treated. The animals in a group did not exhibit any decline in blood glucose profile while the others in that group did are likewise regarded as non-responders. During the experiment, food but not water was withdrawn from the cages [35].

(D) Using a high-fat diet-fed rat model with a low dosage of STZ:

The model is appropriate for pharmacological screening and matches the natural history and metabolic features of type-2 diabetes in humans. The rats get a single injection of STZ (30mg/kg body weight), coupled with a high-energy meal consisting of 20% sucrose and 10% fat. After four weeks, changes in body weight are noted, and established techniques are used to assess blood levels of glucose, TG, TC, and LDL. According to the findings, type-2 diabetes may be successfully induced by modifying the associated gene expressions in key metabolic organs when low dosage STZ and a high-energy diet are combined [37,38].

1. Alloxan-induced diabetes:



Chemical structure of Alloxan. (fig.2)

Mesozalylurea, 2, 4, 5, 6-tetraoxohexa hydro pyrimidine, and pyimidinetetrone are other names for alloxan. It is a derivative of uric acid and is quite unstable in water with a pH of 3, but it is somewhat stable at pH 3. In a cyclic redox reaction involving dialuric acid(fig.3), the reduction product of alloxan, and reactive oxygen species are produced. Hyaluronic acid undergoes autoxidation, which produces superoxide radicals, hydrogen peroxide, and ultimately, hydroxyl radicals in a final iron-catalyzed chemical step. These

hydroxyl radicals ultimately cause insulin-dependent alloxan diabetes by killing beta cells, which have a particularly weak capacity to fight off oxidative stress [39].
An apprentice who was thrust upon a pathology professor led to the amazing discovery of alloxan diabetes. The Professor (J. Shaw Dunn) had spent most of his life researching the kidney, in particular reno-tubular necrosis. Despite being overworked and discouraged from conducting endocrine research during the war, the apprentice (McLetchie) acquired a passion for the field. During a brief wartime work with the apprentice, Colonel Sheehan (later to be memorialized in Sheehan's syndrome) left him with a vivid account of hypoglycemia linked to post-partum pituitary necrosis. Despite being overworked and discouraged from conducting endocrine research during the war, the apprentice (McLetchie) acquired a passion for the field. During a brief wartime work with the apprentice, Colonel Sheehan (later to be memorialized in Sheehan's syndrome) left him with a vivid account of hhypoglycemialinked to post-partum pituitary necrosis. Alloxan diabetes was created after the apprentice saw that this bebehavioras similar in rabbits that had received alloxan in the hazy hope that it might further wartime research on the Crush Kidney condition [40].

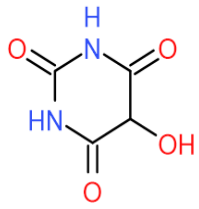


fig.3 structure of Dialuric acid i.e reduction product of alloxan

The dosage of alloxan varies depending on the species of animal, ranging from 40 to 200 mg/kg intravenously or orally for rats, mice, and rabbits, to 50 to 75 mg/kg intravenously for dogs [41]. Animals respond triphasic ally to alloxan. Stage I-early hyperglycemia of brief duration (approximately 1-4 hours) brought on by an abrupt stop or reduction in insulin secretion and direct effects of glycogenolysis on the liver. Hyperglycaemia phase of stage II, which can continue up to 48 hours and frequently results in convulsion and death. Animals with fully established alloxan diabetes histologically show only a few ß-cells, if any, and are in stage III-chronic diabetic phase as a result of insulin deficiency. Exogenous insulin quickly returns blood glucose levels to normal [42].
Another study examines the histological deviations brought on by rabbits' chronic alloxan-induced diabetic Mellitus. By administering four doses of alloxan intraperitoneally at weekly intervals in the form of 80 mg/kg body weight each dose, following a 12-hour fast, New Zealand white male rabbits were experimentally trained to develop diabetes mellitus.
The pancreas, kidneys, lungs, heart, and brain of diabetic rabbits all showed histomorphology changes. The histo-anatomical changes become more severe and affected practically all bodily organs as uncontrolled diabetes progressed. However, very few alterations were seen in the gastrointestinal system, and there was an increase in the vulnerability of the gastric mucosa to yeast cell growth in the stomach [43].
According to a different study, magnesium and alloxan have different effects on the rats' plasma-free fatty acids. In the study, 28 rats were administered intraperitoneal alloxan (120 mg/kg), and after 72 hours, measurements of plasma glucose levels showed that diabetes had been induced. A considerable increase (751.25 mM) in plasma-free fatty acids was observed as compared to the control group (286.68 mM). However, the amount of magnesium in red blood cells in diabetic rats was much lower than in the control group, falling from 7.18 mg/dL to 4.89 mg/dL. The study's findings demonstrated an

antagonistic link between red blood cell magnesium and plasma-free fatty acids in diabetes conditions.

As a result, monitoring the magnesium content of red blood cells after the onset of diabetes may be useful for managing the illness [44].

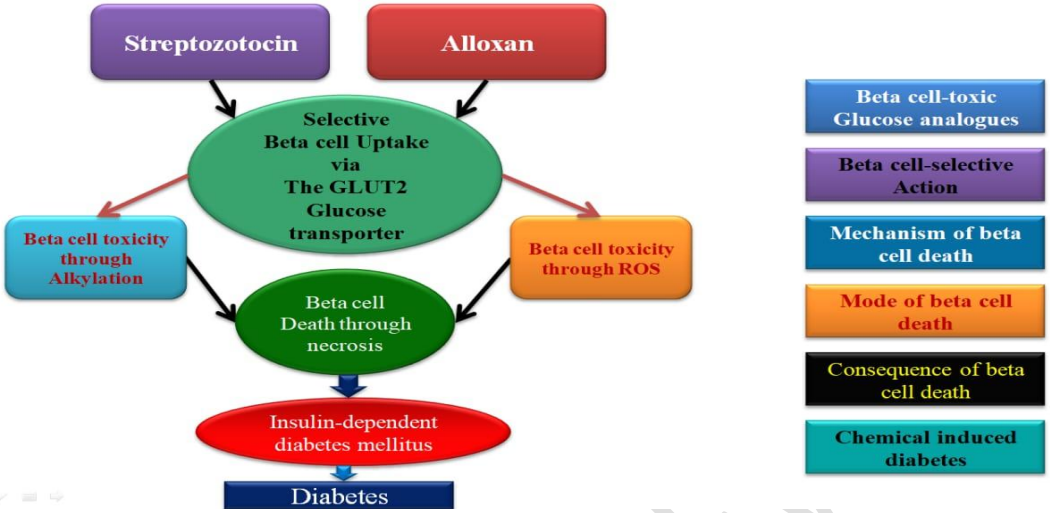


Fig 4 . A visual representation of streptozotocin and alloxan's damaging effects on beta cells.

Table no.1: Classification of numerous animal models for diabetes.

S.no	Type of models	Non-obese	Obese
1.	Animals that be diabetic spontaneously or genetically	1. ALS/Lt mouse, 2. NOD mouse (nonobese diabetic mouse), 3. a mutant mouse from Akita, 4. GK rat, 5. Cohen diabetic rat 6. Torri C57BL/6 rats (Nonobese), eSS-rat, 7. BB rat (Bio Breeding), 8. WBN/Kob rat, and 9.eSS-rat	1. ob/ob mouse, 2. CBA/Ca mouse, 3. the dB/DB mouse 4. KK mouse 5. Mouse KK/Ay, 6. NZO mice 7. The mouse NONcNZO10, 8. the TSOD mouse 9. The M16 mouse 10. Rat BHE, 11. WDF/Ta-fa rat and 12. Zucker fatty rat (Winster fatty rat), 13. ZDF rat. 14. SHR rats with obesity 15. rat SHR/N-cp, 16. JCR/LA-cp rats, 17.OLETF rats, and 18. An overweight rhesus monkey
2.	Diabetic animals caused by diet or nutrition		C57/BL 6J mouse, spiny mouse, and sand rat are the first three.
3.	Animals that have been chemically	1. Low-dose ALX or STZ adult rats,	Obese mice treated with GTG

	made diabetic	2. Neonatal STZ rats, and mice	
4.	Diabetes transgenic or knockout animals	1. PPAR-g knockout mice with tissue-specific IRS-1, IRS-2, GLUT-4, and PTP-1B mutations, 2. mice deficient in GLUT or glucokinase 2, 3. The rat that had human islet amyloid polypeptide expressing (HIP), 4. NOD mice with murine Hsp60 transgenes, and 5. mice with RIP-LCMV transgenes.	gamma-3 receptor-deficient mice and uncoupling protein-deficient mice (UCP1)
5.	Animals made diabetic by a virus	1. Male ICR Swiss mice (prone to the encephalomyocarditis virus D variant) (EMC-D), 2. Male adult C3H/HeJ mice (Resistant to EMC-D), 3. mice DBA/2 (Susceptible to NDK25, a variant of encephalomyocarditis virus which has been cloned from the M variant of encephalomyocarditis virus)	

ALS stands for alloxan sensitive; ALX for alloxan; BB stands for bio breeding; C3H/HeJ stands for lipopolysaccharide response locus; DBA stands for dilute brown non-agouti; GLUT stands for glucose transporter; GK stands for goto Okazaki; GTG stands for gold thioglucose; ICR stands for impaired cytokine response; IRS stands for insulin receptor substrate; JCR stands for James C. Russell, KK for Kuo Kondo, KK/Ay for yellow KK, and NZO for New Zealand. TSOD stands for Tsumara Suzuki obese diabetes; PPAR stands for peroxisome proliferator-activated receptor; PTP stands for phosphotyrosine phosphatase; SHR/N-cp stands for spontaneously hypertensive rat/NIH-corpulent; STZ stands for streptozotocin, and ZDF stands for Zucker diabetic fatty.

3) **Gold thioglucose mouse model for obese diabetics:**

Mice can develop type-2 diabetes with obesity by intraperitoneally injecting goldthioglucose (GTG) at a dosage of 150–350 or 200 mg/kg. After receiving a GTG injection, the animal gradually develops fat, hyperinsulinemia, hyperglycemia, and insulin resistance over 16 to 20 weeks. The ventromedial hypothalamus cells are specifically where the GTG is delivered, where it generates necrotic lesions that lead to the emergence of hyperphagia and obesity. Additionally, it causes anomalies that are qualitatively comparable to those seen in genetically obese mice (ob/ob), including an increase in body lipid, hepatic lipogenesis and triglyceride production, increased adipose tissue lipogenesis, and a reduction in the metabolism of glucose in muscle. Additionally, it demonstrates several molecular flaws in connection to the injection of insulin signalling pathways [45,46].

4) **Model of diabetes caused by atypical antipsychotics:**

It is no longer a surprise that the prescription of atypical drugs is also linked to the appearance of severe metabolic derangement in patients, in addition to the therapeutic improvement over first-generation antipsychotics.
These conditions, which enhance a patient's risk of cardiometabolic illnesses, include glucose dysregulation, insulin resistance, hyperlipidemia, weight gain, and hypertension. Careful consideration must be given to the connection between antipsychotic medications and diabetes [47]. Diabetes is known to be more common in people with schizophrenia than in the general population. One research in this series looked into the diabetogenic effects of a range of antipsychotic medications, both atypical and typical. The hyper insulinemic-euglycemic and hyperglycaemic clamp techniques have been used to assess healthy animals after acute treatment with clozapine (10 mg/kg), olanzapine (3.0 mg/kg), risperidone (1 mg/kg), ziprasidone (3 mg/kg), or haloperidol (0.25 mg/kg). By slowing the pace of glucose infusion and boosting hepatic glucose synthesis, clozapine and olanzapine had an immediate and strong impact on insulin sensitivity. Risperidone lowered peripheral glucose consumption, similar to clozapine and olanzapine. Insulin sensitivity was unaffected by ziprasidone or haloperidol in any substantial way. Clozapine and olanzapine reduced beta cell activity in the hyperglycaemic clamp, which was demonstrated by a reduction in insulin production. Results show that antipsychotic drugs affect metabolic parameters right away, and different atypical antipsychotics have different propensities for producing acute metabolic side effects [48].

5) **Several chemically-induced diabetogenic animal models:**

Drugological features have been studied using the dithizone-induced diabetes model. When zinc interacts with organic substances in the islets of Langerhans, the result is the loss of islet cells and the development of diabetes. The triphasic glycaemic response is caused by the injection of dithizone at dosage levels between 50 and 200 mg/kg. After two hours, initial hyperglycemia will be seen, followed by eight hours of normoglycemia that lasts up to 24 hours. After 24-72 hours, another instance of persistent hyperglycaemia is seen [49].
In a different research, the impact of sirolimus on rat pancreatic islet dysfunction brought on by cyclosporine is discussed. The sirolimus medication led to a dose-dependent rise in blood glucose levels. In comparison to rats treated with cyclosporine A, the combination of sirolimus and cyclosporine increased blood glucose levelshemoglobinin A1c levels, and the HOMA-R [fasting insulin (mU/mL) fasting glucose (mmol/L) /22.5] index while lowering plasma insulin concentrations, insulin immunoreactivity, and pancreatic beta islet cell mass. The study's findings showed that sirolimus causes diabetes and exacerbates the pancreatic islet dysfunction caused by cyclosporine [50]. Additionally, a diabetic model accelerated by cyclophosphamide has been observed. The function of IL-1 in the cyclophosphamide-accelerated form of diabetes was examined by Cailleau et al. Anti-IL-1 Ab was administered twice weekly to male mice that were not diabetic and had had cyclophosphamide injections at a dose of 200 mg/kg. However, only 34% of mice given 0.25 mg of anti-IL-1 Ab developed diabetes [51].
B- surgery to cause diabetes
This technique involves performing a total or partial pancreatectomy on the animals used to induce type-1 or type-2 diabetes, depending. The diabetic dog model developed by Oskar Minkowski by surgical total pancreatectomy was historically thought to be the first animal model of diabetes and is now only sometimes used for research [52].

Few scientists have used this paradigm to investigate how natural compounds affect animals including rats, pigs, dogs, and primates [53,54]. However, partial pancreatectomy and/or combination procedures on animals, particularly non-rodents, are occasionally used in the examination of diabetes for some specialized investigations as detailed below.

- **Non-obese duodenal-jejunal bypass T-2 DM:**

In Goto-Kakizaki rats, a rodent model of non-obese T-2 DM, this approach has been demonstrated to reverse type-2 diabetes (T-2 DM). Sham procedures have been carried out on non-diabetic Wistar-Kyoto rats and Goto-Kakizaki rats. Oral glucose tolerance was assessed two weeks after the duodenal-jejunal bypass, and skeletal muscle insulin-induced signal transduction and glucose disposal were assessed three weeks later. The research established that proximal small intestine bypassing does not improve skeletal muscle glucose absorption. Goto-Kakizaki rats' absence of skeletal muscle insulin resistance raises the question of whether this animal model is suitable for studying the causes and cures of T2 DM. Additionally, bypassing the foregut may have varied results in T-2 DM patients and other animal models of the disease [55].

- **Non-obese partial pancreatectomized diabetic animals:**

Non-obese partial pancreatectomy diabetic animals have been described in a variety of animal species, including dogs, pigs, rabbits, and rats [56,57]. Partial pancreatectomy is performed as a 70 or 90 percent (often 90 percent) pancreatic dissection in animals. It has also been reported in an animal model in which a portion of the pancreas became diabetes due to a nearly complete loss of insulin-secreting B cells, while the other portion of the gland remained normal. In rabbits, the pancreas's body and tail are connected by a vascular clamp, which blocks the blood to the tail. Following an intravenous injection of alloxan (200 mg/kg), dextrose (0.5 g/kg) was administered four minutes later. The clip was removed after 2 minutes. In the first postoperative week, 50% of the animals perished from surgical complications or alloxan-induced liver and kidney damage. The survivors, who were not metabolically diabetic, perished 4 to 12 weeks following surgery. The B cells were almost completely absent, while the A, D, and PP cell populations in the head and body of the pancreas were normal.
The pancreatic tail's islets looked to be in perfect health. This paradigm is thought to be useful for researching how locally generated insulin affects pancreatic exocrine function in animals with normal metabolisms [58]. The experimental setup enables the evaluation of the compound's impact on insulin secretion and resistance.
Animals who undergo pancreatectomy together with chemical treatments like alloxan and STZ develop a persistent type of diabetes mellitus. Combination treatment decreases the interventions required to sustain a pancreatectomized animal, such as enzyme supplementation while reducing the organ damage caused by chemical induction [59]. Another model for the stable form of type 2 diabetes has recently been developed using Balb/c mice treated with NAD (350 mg/kg) and STZ (200 mg/kg) with a 50% partial pancreatectomy [60].
Additionally, by combining bilateral electrolytic lesions of VMH and feeding the animal a high fat and high sugar diet, researchers were able to create the VMH dietary obese diabetic rat, which mimics type-2 diabetes without reducing pancreatic beta cell mass. Severe obesity, hyperinsulinemia, hypertriglyceridemia, insulin resistance, decreased glucose tolerance, mild to severe fasting hyperglycemia and poor modulation of insulin secretory response despite extremely high insulin secretory capacity are some of its hallmarks. Intriguingly, the VMH

lesioned rats exhibit considerable hyperphagia while having higher leptin levels (leptin resistance) [61].
Major surgery and a high risk of animal infection, adequate postoperative analgesia and antibiotic administration, supplementation with pancreatic enzymes to prevent malabsorption, and loss of pancreatic counter-regulatory response to hypoglycaemia are some of the limitations of surgically induced diabetes.

6. **Genetically induced diabetic animal model**

Animals having one or more genetic mutations that are passed down from generation to generation (such as dB/DB mice) can produce spontaneously diabetic individuals, as can animals chosen from non-diabetic outbred animals by repeated breeding over multiple generations [BB rat, T sumara Suzuki Obese Diabetes mouse]. These animals often inherit diabetes as single-gene or multiple-gene abnormalities, as in the case of KK, dB/DB, or Zucker fatty mice and rats. The metabolic characteristics are caused by a single gene deficiency (monogenic), which can be recessive or dominant (e.g., diabetes or dB/DB mice, Zucker fatty rat) or have a polygenic origin (e.g., Kuo Kondo (KK) mouse, New Zealand obese mouse). Even though some subtypes of diabetes do exist with clearly defined causes [for example, maturity-onset diabetes in youth caused by a glucokinase gene defect] and this single gene defect may only occasionally cause type-2 diabetes, the majority of people who develop type-2 diabetes do so as a result of an interaction between environmental factors and multiple gene defects. Consequently, as compared to monogenic animals, polygenic animals better reflect the human state [62,63].

1. Diabetic fatty rat Zuckerr

These were produced by interbreeding a substrain of leptin receptor-deficient (fa/fa) rats that showed hyperglycaemia. Normal islet architecture is disrupted, ß-cell degranulation is enhanced, and ß-cell death is elevated in the Zucker diabetic fatty rat. Between 7 and 10 weeks of age, when the animals in these strains all acquire obesity, insulin resistance, and overt NIDDM, they reach an average plasma glucose level of more than 22 mm [64].
Male Zucker diabetic fatty (mZDF) rats naturally acquire type 2 diabetes, but females only become diabetic when fed a high-fat diabetogenic diet, according to different research (HF-fZDF). The study looked at whether this sex gap may be explained by variations in liver function. To identify those elements that could be protective in females, non-diabetic obese mZDF or HF-fZDF rats were compared to their hepatic molecular profiles. The study established that in ZDF rats, hepatic sex differences may play a role in the sex-based development of diabetes [65]. To research poor wound healing, however, the Zucker diabetic fatty rat with a mutation in leptin receptors may be a promising option. Zucker diabetic fatty rats are suggested as a novel model for research into defective healing because they show deficiencies in wound-size reduction, inflammatory response, tissue organization, and connective tissue turnover [66].

2. Goto-Kakizaki rat:

The Goto-Kakizaki (GK) rat, which is frequently used to investigate type-2 diabetes, provides a genetic model of the disease and exhibits significantly impaired insulin production that causes basal hyperglycaemia. In Japan in 1973, Goto and his colleagues developed the GK rat via selective inbreeding of Wistar rats with impaired glucose tolerance. This process was repeated over multiple generations. Non-obesity, moderate but stable fasting hyperglycaemia, hyperinsulinemia, normolipidemic, decreased glucose tolerance, which manifests in all

animals at 2 weeks of age, and an early start of diabetes sequelae are its defining characteristics. However, it is still unclear what the pancreatic islets of Langerhans in GK rats look like morphologically [67].
Using immunohistochemistry and electron microscopic methods, Momose et al. reported GK rats. We killed GK rats at 7, 14, and 35 weeks of age. In animals older than seven weeks, structural islet alterations were not seen.
GK rats that were 14 and 21 weeks old, however, showed histological islet alterations. Islet shape generally changed, and -cell responses to anti-insulin seemed to be diffusely decreased. According to electron microscopy, there were fewer so-called -granules and more immature granules than before. The Golgi apparatus of -cells was grown, and the cisternae of the rough endoplasmic reticulum were dilated, showing the cells' hyperfunction. According to this study, more complex cellular processes than simple -cell malfunction and/or degeneration are what lead to insulin insufficiency in GK rats [68].
One of the most well-known animal models for examining the relationship between changes in beta cell mass and the incidence of type 2 diabetes and diabetic consequences is the GK rat (particularly diabetic nephropathy). On the other hand, the literature only contains a relatively small number of research on drug testing that used this approach [69].

3**.LEW 1WR1 rats:**

Mordes, J.P. et al. have described a brand-new rat model of autoimmune diabetes that manifested in a major histocompatibility complex congenic LEW rat. The onset of spontaneous diabetes in LEW occurs at a median age of 59 days. 2 percent cumulative frequency in 1WR1 rats (RT1u/u/a). Hyperglycaemia, glycosuria, ketonuria, and polyuria are symptoms of the illness. In contrast to - and -cell populations, which are protected, the islets of severely diabetic rats lack -cells, according to the study. The proportion of ART2+ regulatory T cells in the peripheral lymphoid phenotype is normal. The LEW.1WR1 rat is not vulnerable to spontaneous thyroiditis but is sensitive to collagen-induced arthritis. A novel model animal with a genetic vulnerability to both autoimmune diabetes and arthritis that can be increased by environmental disturbance is the LEW.1WR1 rat [70].

4. **NONcNZO10 mouse**:

The NZO strain was developed over several generations by selective inbreeding, with the parents choosing the colour of their agouti coats. It is a polygenic model of obesity and diabetes. It demonstrates a polygenic syndrome that includes insulin resistance, modest hyperinsulinemia, poor glucose tolerance, hyperphagia, obesity, and mild hyperglycaemia. body mass attributed to hyperphagia, rises quickly during the first two months of infancy. Leptin resistance and hyperleptinemia, which may NZO mice, a mouse model of metabolic syndrome, have been used to investigate the causes of hyperphagia [71]. In NZO mice, obesity develops regardless of the amount of sucrose or fat in the diet or the kind of fat. However, the amount of dietary fat does not increase adiposity and instead hastens the onset of diabetes [72]. A fundamental early impairment in the development of diabetes has been identified as decreased glycogen synthase activity in the liver [73].

5. C57BL/6J mice:

By simply providing high-fat feed to non-obese, non-diabetic animals with type-2 diabetes, created in Japan, the C57BL/6J mouse strain is presently offered by Jackson Laboratory in Bar Harbor. It is characterized by severe obesity, hyperinsulinemia, insulin resistance, and glucose intolerance [74]. Additionally, they show pronounced fasting and basal

hyperglycaemia in contrast to the C57BL/6J (ob/ob) mice's normal basal glucose level. When introduced to high-fat diets after weaning, C57BL/6J (B6) mice become very obese and acquire diabetes, but A/J animals are more likely to be resistant to both conditions. Obesity directly affects how severe diabetes is, and diabetes can be reversed by cutting back on dietary fat [75].
These mice treated with the oral active inhibitor of dipeptidyl peptidase-IV (LAF237) are demonstrated to have corrected glucose tolerance in conjunction with increased insulin production, demonstrating further evidence of its efficacy for drug testing [76].

6. db/db mice:

Originally generated from an autosomal recessive mutation on chromosome 4 in mice of the C57BL/KJ strain originating from Bar Harbor, Maine, the DB/DB (diabetes) mouse has been renamed leopard. The DB gene, which codes for the leptin receptors, was shown to be the source of the mutation in this diabetic animal. These mice acquire obesity, hyperglycaemia, hyperinsulinemia, and insulin resistance within the first month of life and hyperinsulinemia and hyperglycaemia thereafter, with a peak between 3 and 4 months of age [77].
These mice are naturally hyperphagic insulin over secretors. After that, animals experience ketosis, gradually lose body weight, and die after 8–10 months [45]. The examination of type 2 diabetes and diabetic dyslipidaemia as well as the testing of medications like insulin mimetics and insulin sensitizers have both been widely and frequently conducted using DB/DB mice [78].
According to reports, DB/DB and ob/ob mice have different platelet functions and coagulation patterns. Do not exhibit a hypercoagulable condition resembling that of type-2 diabetic people [79].

Virus-induced diabetic animal model

Epidemiologists generally agree that in recent decades, the incidence rate of type-1 diabetes has increased globally. Although the reason for this increase is uncertain, epidemiological studies point to the possibility of environmental variables, particularly viral infections. The idea that viruses cause illness via processes connected with innate immune regulation is supported by new data from animal models. A parvovirus infection causes islet loss in the Bio Breeding Diabetes Resistant rat by activating the toll-like receptor 9 (TLR9) signalling pathway [80].
Viruses attack and kill pancreatic beta cells to cause diabetes mellitus. A less infectious or cytologic version causes equivalent harm to the -cells by triggering an immunological auto reaction. A less infectious or cytologic version causes equivalent harm to the -cells by triggering an immunological auto reaction.
RNA picornaviruses, Coxsackie B4, encephalomyocarditis (EMC-D and M variations), Mengo-2T, reovirus, and lymphocytic choriomeningitis are some of the human viruses that have been exploited to cause diabetes [81,82].
The development of T-1D may be influenced by enteroviruses, such as coxsackievirus B4 (CV-B4), according to data from retrospective and prospective epidemiological investigations. It has also been demonstrated that, as compared to control participants, at-risk groups such as siblings of diabetes patients, those who acquire anti-cell autoantibodies or T-1D, and newly diagnosed diabetic patients have a much higher prevalence of enterovirus infections. The discovery of CV-B4 in the pancreas of diabetes patients lends credence to the idea that the virus and the condition are related [83].

The pathogenic mechanisms of infection that can cause -cell degeneration, such as direct virus-induced -cell lysis, molecular mimicry, bystander activation, and viral persistence, have been made clearer by studies carried out in vitro and in vivo in animal models, which have improved our understanding of the role of CV-B4 in T1D. Even while there are compelling indications that enteroviruses play a significant role in the pathophysiology of T-1D, a causal relationship between these pathogens and the disease has not yet been demonstrated [84].

Oral glucose loading animal model

Because the animal's blood glucose level is briefly elevated without causing pancreatic harm, this technique is sometimes referred to as physiological induction of diabetes mellitus. It is widely used to identify gestational diabetes, diabetes mellitus, and impaired glucose tolerance. In the clinical world, it is known as the oral glucose tolerance test. Indicators of insulin production and insulin sensitivity can be determined from simultaneous measurements of plasma glucose, insulin, or, less commonly, C-peptide levels. These indicators are helpful for evaluating changes in glucose metabolism as well as for forecasting the transition from normal glucose tolerance to impaired glucose tolerance or type 2 diabetes. The oral disposition index, a recently created statistic that combines insulin secretion and insulin sensitivity, is becoming more and more popular.
More and more people are interested in the oral disposition index, a newly developed metric that combines insulin secretion and insulin sensitivity. especially for the type-2 diabetes prognosis [85].
Another study indicated that at the determination of the maximum achievable decrease of individual variability by Comparing the diagnostic value of various intravenous and oral glucose doses as well as the iv GTT and oral GTT following the same load. The authors initially carried out an intravenous glucose tolerance test (GTT) using 1 g of glucose per kilogram of body weight and an oral Normal (non-anesthetized) adult (F 6-7) inbred rats and mice were subjected to GTT with 1, 2, and 4 g/kg, and these tests were then repeated the same rats and mice were used, with identical loads, and the B-Cell damage following intravenous alloxan injection varied in severity. Results showed that a higher load (4 g/kg orally) was required to reveal latent ("chemical") diabetes, and that the assimilation constant of an intravenous GTT was diabetic sooner than the criteria of an oral GTT after an equal oral load (1 g/kg), and that the shape of the GTT curves is crucial for the diagnosis of early stages of diabetes in addition to their absolute values [86].

Diabetes caused by insulin antibodies

Anti-insulin antibodies insulin induced when guinea pigs are given CFA and bovine insulin. Rats receiving an intravenous injection of guinea pig anti-serum between 0.25 and 1.0 ml have a dose-dependent rise in blood sugar levels up to 300 mg/dl. This result results from insulin antibodies neutralizing endogenous insulin. It continues for as long as the antibodies are still able to react with insulin that is still in the bloodstream. Ketonemia, ketonuria, glycosuria, and acidosis accompany high dosages and extended administration [87].

Table no.2: Numerous groups of diabetic animal models have benefit and drawbacks.

S. no.	Type of models	Benefits	Drawbacks
1.	Animals that be diabetic	1. The diverse spontaneous origins of diabetes better	1. When the human ADME system is more sophisticated

	spontaneously or genetically	illustrate the intricate nature of diabetes in humans [94,13,8,5]. 2. A variety of animal techniques are available based on how they start to work or how they work. While some models portray a range of modes of action, including insulin resistance to type II diabetes, other models depict the development of diabetes extremely quickly [92,95,96].	than these models [2,10–12], the animal models are extremely monogenic in addition to demonstrating many modes of action. 2. It is difficult to maintain post-diabetes maintenance in animals since there aren't many animal models accessible [92,95,96].
2.	diabetic animals caused by diet or nutrition	1. The experiment is economical since diabetes is brought on by diet or nutrition, which lowers the expense of the study. 2. Less harmful to essential organs and safer than chemicals.	1. A lengthy period is required because animals are fed an excess of their diet to raise blood sugar levels.
3.	Animals that have been chemically made diabetic	1. By targeting pancreatic beta cells only, the toxicity prevents other problems. 2. Given that the proper dosages of STZ and ALX were previously reported [3,23], the mortality rate is comparatively low.	1. Due to the high rate of beta cell production, the therapy is less durable and reversible. 2. Another significant issue is toxicity in other crucial organs.
4.	Diabetes transgenic or knockout animals	1. There is no toxicity of other important organs in the model [136,4]. 2. By solely eliminating the pancreatic beta cells, the model may be created quickly and extremely selectively [136,4].	1. The entire business must be set up at a high cost. 2. The procedure is technically challenging to conduct [91,92,10,93]. 3. The postoperative hormone and antibiotic supplements are quite pricey and demand a high level of supervision [91,92,10,93].
5.	Animals made diabetic by a virus	1. The model is very focused and able to provide substantial insight into a single gene.	1. It was expensive and complicated to construct the model.

***In-vitro* models**

In earlier research, glucose absorption into isolated fat cells from rat epididymis was used to evaluate insulin-like activity. This study made extensive use of rat epididymal fat pad adipose tissue. The glucose absorption was determined by the glucose content in the medium or the oxygen consumption in Warburg vasculature after the epididymal rat adipose tissue was cultured in media containing glucose. Incubating rat epididymal adipose tissue allowed

researchers to determine that the rate of glucose transport across the cell membrane largely regulated the amount of glucose absorption. A new sort of experiment was conducted using the radio-labelled glucose in which 14CO2 was captured and measured. This method [101,102] was used to measure the amount of 14CO2 produced from the iodine-labeled 14C glucose. To calculate the total gas exchange for the purpose of detecting the little amount of insulin, a manometric test was used [103,104].
This strategy received some modification in following research. Isolated fat cells, 3T3-L1 adipocytes, and freshly cultured adipocytes have all been found to be especially helpful for analysing insulin-like action utilising in vitro models [105].

Table no.3: A comparison of alloxan and streptozotocin.

S.no	**Property**	**ALX**	**STZ**
1.	A chemistry name	Tetraoxy-2,4,5,6-pyrimidine and 2,4,5,6-pyrimidinetetrone	2-Deoxy-2-([(methylnitrosoamino)carbonyl]amino) D-glucopyranose
2.	Chemical Description	Barbituric acid derivatives and oxygenated pyrimidine derivatives (5ketobarbituric acid)	Glucosamine derivative; glucose (2-deoxyglucose) molecule linked to a cytotoxic methyl nitrosourea moiety (N-methyl-N-nitrosourea)
3.	qualities of chemicals	1. A very hydrophilic, beta cell-toxic glucose analog with a weak acid (partition coefficient = 1.8). 2. Stable at acid pH; chemically unstable (half-life of 1.5 min at pH 7.4 and 37 °C, degrading to alloxanic acid);	1. A beta-cell-toxic, a hydrophilic glucose analog 2. At pH 7.4 and 37 °C, it is rather stable (at least for up to 1 h)
4.	Reactivities of chemicals	1. Thiol reagent that, in the presence of GSH and other thiols, is reduced to dialuric acid 2. A protoxin; by redox cycling with dialuric acid over an extended length of time (more than 1 hour), the intracellular metabolism of this xenobiotic produces lethal ROS. 3. Compound 305, a minor quantity of which is generated, is an unidentified non-toxic alloxan-GSH adduct with a distinctive absorbance at a wavelength of 305 nm.	1. DNA methylating substance 2. Agents that alkylate proteins 3. NONE donor
5.	toxicological type	production of ROS	Alkylation of DNA
6.	Amount(mg/kg)	40–200 rats (i.v./i.p.) 50–200 mice (i.v./i.p.) Rabbit: 100-150 (i.v.) Dog: 50-75 (i.v.)	35–65 (i.v./i.p.) rats i.v./i.p. mice: 100–200 Hamster: 50 (i.p.) Dog: 20-30 (i.v.) Pig: 100–150 (i.v) (i.v.)

			i.v. 50–150 primates)

i.v. stands for intravenous, and i.p. for intraperitoneal.

Conclusion:

In comparison to other models, both in vivo and in vitro models offer benefits. The in vitro models are quicker and more cell-specific for antidiabetic research. Numerous assays might be used to quickly analyse the impacts. The in vivo procedures, however, take longer and cost more money. Furthermore, to conduct in vivo studies correctly and provide consistent results, a great deal of skill is required. The human body system, however, is a complicated system that cannot be fully understood by in vitro tests alone. For a better understanding, it is necessary to assess the effectiveness of sample molecules using an in vivo system. In conclusion, it is possible to advocate using in vitro animal models in particular to study on the specific mechanisms as well as early-stage studies to identify the precise target molecule or receptor. For more research to produce medication and assess toxicity profiles, in vivo models are suggested.

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