Animal models for biological screening of anti-diabetic drugs: An overview

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ABSTRACT

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide thus the disease constitutes a major health concern. Presently, it is an incurable metabolic disorder which affects about 2.8% of the global population. The search for compounds with novel properties to deal with the disease condition is still in progress. This makes the use of experimental models for the disease imperative. The current review has attempted to bring together all the reported models, highlighted their short comings and drew the precautions required for each technique. Type-1 diabetes requires insulin treatment, whereas Type-2 diabetes, which is characterized by insulin resistance, can be treated using a variety of therapeutic approaches. Hyperglycemia is thought to be a primary factor in the onset of diabetes, although hyperlipidemia also plays a role. The major organs active in the regulation of blood glucose are the pancreas, liver, skeletal muscle, adipose tissue, intestine, and kidney. The purpose of this review article is to describe the significance of various animal models available for screening of antidiabetic activity.

Key words: Animal Model, Diabetes Mellitus, Hyperglycemia, Induction, type-2 diabetes
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INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by either the insufficient production or the lack of response to a key regulatory hormone of the body's metabolism, insulin. It can be categorized as Type-1 diabetes [insulin dependent diabetes mellitus (IDDM)] and Type-2 diabetes [non- insulin dependent diabetes mellitus (NIDDM)]. The overall prevalence of diabetes is approximately 10% of the population, of which 90% is Type-2 [1]. The disease is characterized by hyperglycemia, hypercholesterolemia, and hypertriglyceridemia, resulting from defects in insulin secretion or reduced sensitivity of the tissue to insulin (insulin resistance) and/or combination of both. Characteristically, it is a serious endocrine syndrome with poor metabolic control and responsible for increased risk of cardiovascular diseases including atherosclerosis, renal failure, blindness or diabetic cataract [2].

Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes. Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic considerations. Experimental diabetes mellitus is generally induced in laboratory animals by several methods that include: chemical, surgical and genetic (immunological) manipulations [3]. Type-2 diabetic models include the genetically altered Zucker diabetic fatty (ZDF) rats, Otsuka

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Long Evans Tokushima fatty (OLETF) rats, Goto Kakizaki (GK) rats, spontaneously diabetic Tori (SDT) rats, *ob/ob+/+* mice, and *db/db* mice, which feature insulin resistance and reduced β-cell mass [4]. The large number of different models developed for different traits and insufficient characterization of some models make it difficult to choose the right model for a given study (including pharmacological screening) and at times leading to misinterpretation of data or even to the wrong conclusions. It is also very important to select appropriate animal model for the screening of new chemical entities (NCEs) and other therapeutic modalities for the treatment of type 2 diabetes [5].

The aim of the present review is to piece together various experimental models, including Type-1, Type-2 with emphasis on documented models for secondary complications of diabetes mellitus, to assess the merits and demerits of each model and highlight the precautions needed to avoid erroneous results during the applications of these models.

Animal Models for Type-1 & Type-2 Diabetes
I- Chemically induced diabetes-
Chemical agents which produce diabetes (diabetogenic agent) can be classified into three categories, and include agents that: specifically damage ß- cell, cause temporary inhibition of insulin production and/ or secretion and diminish the metabolic efficacy of insulin in target tissue. The following text is summarizes the models based on use of diabetogenic agents.

1) Streptozocin (STZ) induced diabetes:
Streptozocin (STZ) is a glucosamine-nitrosourea compound that has been in clinical trial since 1967. It was formerly designated streptozotocin, but its name has been shortened by the US Adopted Names Council. A New Drug Application has been filed with the US Food and Drug Administration by The Upjohn Co (Kalamazoo, MI) for marketing under the trade name Zanosar. Approval of this application is imminent [6]. STZ induces diabetes in almost all species. Diabetes can be induced by STZ either by either by single injection of STZ or by multiple low dose injection of STZ. STZ is the most commonly used drug for induction of diabetes in rats [7].

Intra-venous injection of 60mg/kg dose of streptozotocin in adult wistar rats causes swelling of pancreas followed by degeneration of Langerhans islet beta cells and induces experimental diabetes mellitus in the 2-4 days. Three days after degeneration of beta cells, diabetes was induced in all animals. Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological effects in beta cells which probably intermediates induction of diabetes [8].

While rodents have been extensively used as the animal species, other animals have also been utilized. In a study, the induction of diabetes in New Zealand male rabbits was accomplished by single intravenous injection of streptozotocin (65mg/kg body weight). The study was designed to investigate the biochemical and histomorphological changes occurring due to streptozotocin-induced diabetes mellitus in rabbits [9].

Insulin-mediated glucose metabolism has investigated in streptozotocin (STZ)-treated diabetic pigs to explore if the STZ-diabetic pig can be a suitable model for insulin-resistant, type-2 diabetes mellitus. This study concluded that a slow infusion of STZ (130 mg/kg) in pigs on a low-fat diet induces the characteristic metabolic abnormalities of type-2 diabetes mellitus and its sensitivity to oral metformin therapy. It is therefore a suitable humanoid animal model for studying different aspects of metabolic changes in type-2 diabetes mellitus. Insulin resistance in STZ-diabetic pigs is most likely secondary to hyperglycemia and/or hyperlipidemia and therefore of metabolic origin [10].

Streptozotocin (STZ), preferentially toxic to pancreatic beta cells, is commonly used to model Type-1 diabetes mellitus (DM) in numerous species, including nonhuman primates. A study diabetes mellitus was induced in vervet monkeys (*Chlorocebus aethiops*) by intravenous administration of either 45 (n = 8, STZ-45) or 55 mg/kg STZ (n = 12, STZ-55) and ten control (CTL) monkeys received saline. Exogenous insulin requirements increased rapidly for four weeks; STZ-45 insulin dose stabilized thereafter while STZ-55 doses continued to increase through 16 weeks. Glucose tolerance testing and arginine-stimulated insulin secretion confirmed 80-90% reduction in pancreatic beta cell function in both groups. Body weight was reduced in all STZ monkeys, with return to baseline only in STZ-45 at 16 weeks. Elevated blood urea nitrogen and creatinine levels were noted in the STZ-55 group. Alkaline phosphatase also increased with STZ-55 (p < 0.05 versus control) whereas STZ-45 alkaline phosphatase elevation

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was resolved by the end of the study. Red cell parameters were reduced in all STZ monkeys, but more severely in the STZ-55 group. This model demonstrated that diabetes mellitus can be induced and maintained in vervets with a single dose of STZ. The lower dose of STZ (45 mg/kg) significantly improved the toxicity profile without altering efficacy in inducing diabetes mellitus. Finally, sufficient time following induction is recommended to resolve transient renal, hepatic and hematologic parameters [11].

Severe IDDM (insulin-dependent diabetes mellitus) has been produced in the musk shrew (*Suncus murinus, Insectivora*) by a single high dose intraperitoneal injection of 100 mg/kg body weight) of STZ injection. The data of this model indicated that the IDDM in shrew, induced by high doses of STZ, is a unique model characterized by fatty liver and hyperlipidemia and may be useful for studying lipid metabolism of IDDM [12]. Literature also reports STZ induced diabetes cattle, cows in a dose size of 75-150 mg STZ per kilogram of body weight. Alternative dosages and methodologies should be considered in future to induce diabetes in cattle using STZ [13].

(A) Neonatal Streptozotocin induced diabetes rat model (n-STZ)
The n-STZ model (with alteration of dose and day of STZ injection) exhibits various stages of Type-2 diabetes mellitus such as impaired glucose tolerance, and mild, moderate and severe glycemia [14]. Single dose of STZ 100 mg/kg i.p.; to the one day old pup and 120 mg/kg i.p. to the two, three, or five day old pups induces diabetes. The β cells in n-STZ rats bear a resemblance to insulin secretory characteristics found in patients with Type-2 diabetes mellitus. Thus the n-STZ model can be considered as one of the suitable animal models of Type-2 diabetes mellitus [15].

(B) Nicotinamide-Streptozotocin (NAD-STZ) induced diabetic model
This model has the advantage of the partial protection exerted by suitable dosages of nicotinamide against the β-cytotoxic effect of streptozotocin (STZ) to create a new experimental diabetic syndrome in adult rats that appears closer to NIDDM than other available animal models with regard to insulin responsiveness to glucose and sulfonylureas. 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 [16]. Non insulin dependent diabetes mellitus (NIDDM) was induced by a single intraperitoneal injection of STZ (60mg/kg) and NAD (120mg/kg) to rats. NAD is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass producing type-2 diabetes. Therefore, this model is seen as an advantageous tool for investigation of insulinotropic agents in the treatment of type-2 diabetes [17].

(C) Sucrose-challenged streptozotocin-induced diabetic rat model (STZ-S)
This model screened in vivo antidiabetic activity in sucrose loaded model (SLM) male albino rats. Charles Foster/Wistar strain rats of average body weight 160 ± 20 g weight were used. STZ dissolved in 100 mM citrate buffer, pH 4.5 and calculated amount of the fresh solution was injected intraperitoneally to overnight fasted rats (60 mg/kg). Blood glucose levels were checked 48 h later by glucostrips and animals with blood glucose values between 144 and 270 mg/dl (8–15 mM) were considered as diabetic. A sucrose load of 2.5 g/kg body weight was given 30 min later. Thirty minutes post sucrose load, blood glucose levels were again checked by glucostrips at 30, 60, 90, 120, 180, 240, 300 min and at 24 h, respectively. Animals not found diabetic after 24 h post treatment of the test sample were termed as non-responders. The animals, which did not show any fall in blood glucose profile in a group while the others in that group, showed fall in blood glucose profile have also considered as non-responders. Food but not water has withheld from the cages during the experimentation [18].

(D) Low dose STZ with high fat diet-fed rat model
The model replicates the natural history and metabolic characteristics of human type-2 diabetes and is also suitable for pharmacological screening. The rats are administered high-energy diet of 20% sucrose and 10% lard along with single injection of STZ (30mg/kg body weight). After 4 weeks changes in body weight are recorded and levels of glucose, TG, TC, LDL in serum are analyzed by standard methods. The results suggested that a combination of low dose STZ and high-energy diet intake can effectively induce type-2 diabetes by altering the related gene expressions in major metabolic tissues [19, 20].

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2) Alloxan induced diabetes:
Alloxan is also called as mesozalylurea, mesoxalylcarbamide 2, 4, 5, 6-tetraoxohexa hydropyrimidine or pyimidinetetrone. It is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. Alloxan generates reactive oxygen species in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defence capacity, and ensure state of insulin-dependent alloxan diabetes [21].

The remarkable discovery of alloxan diabetes came about between a professor of pathology and an apprentice who was foisted on him. The Professor (J. Shaw Dunn) had a lifetime behind him largely dedicated to studies on the kidney and particularly reno-tubular necrosis. The apprentice (McLetchie), despite overburdening duties and discouragement against endocrine research in wartime, developed a passion for endocrine investigation. A Colonel Sheehan (later to be enshrined in Sheehan's syndrome) in a brief wartime collaboration with the apprentice left him with a vivid description of hypoglycemia associated with post-partum pituitary necrosis. The apprentice saw this behavior paralleled in rabbits which had been given alloxan in a vague belief that it would further wartime research on the Crush Kidney syndrome, and so, alloxan diabetes was born [22]. The dose of alloxan varies with different species of animals like rat 40-200mg/kg i.v or i.p., mice 50-200 mg/kg i.v or i.p., rabbit 100-150 mg/kg i.v. and for dogs it is 50-75 mg/kg i.v. [23]. Alloxan causes triphasic response in animals Stage I-early hyperglycemia of short duration (about 1-4 hr) due to a sudden short lasting decrease or cessation of insulin release and direct glycogenolytic effects on the liver. Stage II-hyperglycemia phase lasting up to 48 hrs and often resulting in convulsion and death. Stage III-chronic diabetic phase consequence of insulin lack histologically only a few ß- cells if any are detectable in animals with fully developed alloxan diabetes. Exogenous insulin readily restores normal blood glucose levels [24].

Another study investigates histopathological abnormalities due to prolonged alloxan-induced diabetes mellitus in rabbits. Diabetes mellitus was experimentally induced in New Zealand white male rabbits by intraperitoneal administration of four doses of alloxan as 80 mg/kg body weight at weekly intervals following 12hr fasting. Histomorphological alterations were recorded for pancreas, kidneys, lungs, heart and brain in diabetic rabbits. With the progress of untreated diabetes, the histoanatomical alterations intensified and extended to almost all organs of the body. However, mild changes were observed in gastrointestinal tract with proliferation of yeast in the stomach indicating an increase in the susceptibility of gastric mucosa to yeast cell proliferation [25].

Another investigation reports contrasting effects of alloxan and magnesium on plasma free fatty acids in rats. The study used 28 rats that received alloxan (120mg/kg) intraperitoneally and plasma glucose level measurement after 72 hours demonstrated diabetes induction. Analysis of plasma free fatty acids showed a significant increase (751.25 mM), compared to the control group (286.68 mM). In contrast, the red blood cell-magnesium level showed a significant decrease from 7.18 mg/dL in control group to 4.89 mg/dL in diabetic rats. The results of the study showed an inverse relationship between plasma free fatty acids and red blood cell- magnesium in diabetic condition. Thus analysis of red blood cell- magnesium upon induction of diabetic condition could provide important information for management of diabetes [26].

3) Goldthioglucose obese diabetic mouse model:
Type-2 diabetes with obesity can be induced in mice by intraperitonial injection of goldthioglucose (GTG) in a dose of 150-350 or 200 mg/kg. The animal gradually develops obesity, hyperinsulinaemia, hyperglycemia, insulin resistance over a period of 16- 20 weeks after GTG injection. The GTG is transported in particular to the cells of ventromedial hypothalamus and causes necrotic lesions, which subsequently are responsible for the development of hyperphagia and obesity. It also increases body lipid, hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreases glucose metabolism in muscle, abnormalities that are qualitatively similar to genetically obese mice (*ob/ ob*). In addition, it exhibits many molecular defects in relation to insulin signaling pathways injection [27, 28].

4) Atypical antipsychotic-induced diabetic model:
Besides the therapeutic improvement over first-generation antipsychotics, the fact that prescription of atypical agents is also associated to the emergence of severe metabolic derangement in patients is not a mystery anymore. These include glucose deregulation, insulin resistance, hyperlipidemia, weight gain and hypertension, which put patients at increased risk of cardiometabolic disorders. The relationship between diabetes and antipsychotic drugs

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requires a careful analysis [29]. Patients with schizophrenia are known to suffer from diabetes more often than the general population.

In this series one study has been investigated the diabetogenic effects of a spectrum of antipsychotics, both atypical and typical. Healthy animals have treated acutely with clozapine (10 mg/kg), olanzapine (3.0 mg/kg), risperidone (1 mg/kg), ziprasidone (3 mg/kg) or haloperidol (0.25 mg/kg) and tested using the hyperinsulinemic-euglycemic and hyperglycemic clamp procedures. Clozapine and olanzapine had a rapid and potent effect on insulin sensitivity by lowering the glucose infusion rate and increasing hepatic glucose production. Both clozapine and olanzapine, as well as risperidone, decreased peripheral glucose utilization. Neither ziprasidone nor haloperidol had a significant impact on insulin sensitivity. In the hyperglycemic clamp, clozapine and olanzapine impaired beta cell function as reflected by a decrease in insulin secretion. Results confirm that antipsychotic medications have an immediate impact on metabolic parameters and the various atypical antipsychotics differ in their propensity to acutely induce metabolic side effects [30].

5) Miscellaneous chemical diabetogenic animal models:
Dithizone induced diabetes model have been used in pharmacological aspects. Organic agents (8-(p-toluene-sulfonylamino) quinoline) react with zinc in islets of Langerhans causing destruction of islet cells and producing diabetes. Dithizone injection at a dose level of 50-200 mg/ kg will produce triphasic glycemic reaction. Initial hyperglycemia will be observed after 2h and normoglycemia after 8h, which persists for up to 24 h. Again hyperglycemia is observed after 24-72 h which lasts for longer period of time [31].

Another study describes the effect of sirolimus on cyclosporine -induced pancreatic islet dysfunction in rats. The sirolimus treatment increased blood glucose concentration in a dose-dependent manner. The combined treatment with sirolimus and cyclosporine increased blood glucose concentration, hemoglobin A1c level, HOMA-R [fasting insulin (mU/mL) fasting glucose (mmol/L) /22.5] index and decreased plasma insulin concentration, immunoreactivity of insulin and pancreatic beta islet cell mass compared with rats treated with cyclosporine A. The results of the study demonstrated that sirolimus is diabetogenic and aggravates cyclosporineA-induced pancreatic islet dysfunction [32].

Cyclophosphamide -accelerated model of diabetes has also been reported. Cailleau.*et al* evaluated the role of IL-1β in the cyclophosphamide-accelerated model of diabetes. Non-diabetic male mice injected with 200 mg/kg cyclophosphamide were treated twice weekly with anti-IL-1β Ab. In contrast, only 34% of mice treated with 0.25 mg of anti-IL- 1β Ab became diabetic [33].

II- Surgically induced diabetes-
This method consists of complete or partial pancreatectomy in animals used for the induction of type-1 or type-2 diabetes respectively. Historically, the diabetic dog model discovered by Oskar Minkowski through surgical complete pancreatectomy has been considered to be the first animal model of diabetes and is rarely now used for the investigation [34]. Few researchers have employed this model to explore effects of natural products with animal species such as rats, pigs, dogs and primates [35, 36]. However, partial pancreatectomy and/or combination methods on animals particularly non rodents are at times utilized in the diabetes investigation for some specific studies as described below.

1) Duodenal-jejunal by pass non-obese T-2 DM:
This model has been shown to reverse type-2 diabetes (T-2 DM) in Goto-Kakizaki rats, a rodent model of non-obese T-2 DM. Sham operations have been performed in Goto-Kakizaki and non-diabetic Wistar-Kyoto rats. Two weeks post-duodenal-jejunal bypass, oral glucose tolerance was measured and after three weeks insulin-induced signal transduction and glucose disposal was measured in skeletal muscle. The study proved that bypassing of the proximal small intestine does not increase skeletal muscle glucose disposal. The lack of skeletal muscle insulin resistance in Goto-Kakizaki rats questions whether this animal model is adequate to investigate the etiology and treatments for T-2 DM. Additionally, bypassing of the foregut may lead to different findings in other animal models of T-2 DM as well as in T-2 DM patients [37].

2) Non obese partial pancreatectomized diabetic animals:
Partial pancreatectomy in animals performed as 70 or 90 per cent (usually 90%) dissection of pancreas has been reported in various animal species namely in dogs, pigs, rabbit and also rats [38, 39]. An animal model in which part

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of the pancreas has made diabetic due to almost total loss of insulin-secreting B cells, while the remainder of the gland remained normal has also been reported. In rabbits, a vascular clamp is placed across the junction of the body and tail of the pancreas, thus occluding the circulation to the tail. Alloxan (200 mg/kg) was injected i.v. and 4 min later dextrose (0.5 g/kg) was given by same route. After 2 min the clamp was removed. 50% of the animals died in the first postoperative week of surgical complications or of alloxan-induced toxicity to the liver and kidneys. The survivors were killed between 4 and 12 weeks after surgery and were not metabolically diabetic. They had virtually a complete absence of B cells but a normal population of A, D, and PP cells in the head and body of the pancreas. The islets in the tail of the pancreas appeared entirely normal. This model is considered suitable for studying the effects of locally produced insulin on pancreatic exocrine function in metabolically normal animals [40].The experimental design permits evaluation of the compound effectiveness on both resistance and secretion of insulin.

The use of pancreatectomy in combination with chemical agents, such as alloxan and STZ, produces a stable form of diabetes mellitus in animals. The combination therapy reduces the organ damage associated with chemical induction and minimizes the interventions, such as enzyme supplementation, necessary to maintain a pancreatectomized animal [41]. Recently, another model on stable form of type-2 diabetes has been introduced by combination of 50 per cent partial pancreatectomy along with NAD (350-mg/kg) and STZ (200 mg/ kg) treatment in Balb/c mice [42].

Additionally, VMH dietary obese diabetic rat has been developed by experimental surgical manipulation of genetically normal animals without the reduction in pancreatic beta cell mass, resembling type-2 diabetes, by combining bilateral electrolyte lesion of VMH and feeding the high fat and high sucrose diet to the animal. It is characterized by marked obesity, hyperinsulinaemia, hypertriglyceridaemia, insulin resistance, impaired glucose tolerance, moderate to severe fasting hyperglycaemia and defective regulation of insulin secretory response despite extremely high insulin secretory capacity. It is interesting that significant hyperphagia is observed despite increased leptin levels (leptin resistance) in the VMH lesioned rats [43].

Limitations to surgically induced diabetes include high level of technical expertise and adequate surgical room environment, major surgery and high risk of animal infection, adequate post-operative analgesia and antibiotic administration, supplementation with pancreatic enzymes to prevent malabsorption and loss of pancreatic counter regulatory response to hypoglycemia.

III- Genetically induced diabetic animal model-
Spontaneous diabetic animals of type-2 diabetes may be obtained from the animals with one or several genetic mutations transmitted from generation to generation (e.g. db/db mice) or by selected from non-diabetic out bred animals by repeated breeding over several generation [BB rat, Tsumara Suzuki Obese Diabetes mouse]. These animals generally inherit diabetes either as single or multigene defects as seen in KK mouse, db/db mouse, or Zucker fatty rat. The metabolic peculiarities result from single gene defect (monogenic) which may be due to dominant gene (*e.g.*, Yellow obese or KK/A mouse) or recessive gene (diabetic or db/db mouse, Zucker fatty rat) or it can be of polygenic origin [*e.g.*, Kuo Kondo (KK) mouse, New Zealand obese mouse]. Type-2 diabetes occurring in majority of human beings is a result of interaction between environmental and multiple gene defects though certain subtype of diabetes do exist with well defined cause [*i.e.*, maturity onset diabetes of youth due to defect in glucokinase gene] and this single gene defects may cause type-2 diabetes only in few cases. Therefore, polygenic animals represent the human condition more closely when compared to monogenic animals [44, 45].

1) Zukker Diabetic Fatty Rat:
These arose from the inbreeding of a substrain of fa/fa (leptin receptor-deficient) rats that exhibited hyperglycemia. Zucker diabetic fatty rat is associated with disruption of normal islet architecture, ß-cell degranulation, and increased ß-cell death. In this strain all animals develop obesity, insulin resistance and overt NIDDM between 7 and 10 weeks of age, by which time their average plasma glucose exceeds 22 mM [46].

Another study has evidenced that the Male Zucker diabetic fatty (mZDF) rats spontaneously develop type-2 diabetes, whereas females become diabetic when fed with diabetogenic high-fat diet (HF-fZDF). The study investigated if the differences in liver functions could provide clue to this sex difference. Non-diabetic obese fZDF rats were compared with either mZDF or HF-fZDF for their hepatic molecular profiles, to single out those components that might be protective in the females. The work proved that the hepatic sex differences might contribute to the sex-based development of diabetes in ZDF rats [47].

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However, Zucker diabetic fatty rat, with a mutation in leptin receptors, may be a good choice for studying impaired wound healing. Zucker diabetic fatty rat's exhibit impairments in wound-size reduction, inflammatory response, tissue organization, and connective tissue turnover and are thus proposed as a new model for studying impaired repair [48].

2) Goto-Kakizaki rat:
The Goto-Kakizaki (GK) rat offers a genetic model of type-2 diabetes and displays profoundly defective insulin secretion leading to basal hyperglycemia and is widely used for studying type-2 diabetes. The GK rat, a polygenic model of type-2 diabetes was established by *Goto* and his collaborators through selective inbreeding of Wistar rats with abnormal glucose tolerance repeated over several generations in Japan in 1973. It is characterized by non obesity, moderate but stable fasting hyperglycaemia, hypoinsulinaemia, normolipidaemia, impaired glucose tolerance which appears at 2 weeks of age in all animals and an early onset of diabetic complications. However, the morphological characteristics of the pancreatic islets of Langerhans in GK rats are not fully understood [49]. Momose *et al* have been reported GK rats using immunohistochemical and electron microscopic techniques. GK rats were killed at 7, 14, 21, and 35 weeks of age. Structural islet changes were not observed in 7 weeks old animals. However, animals of 14 and 21 weeks age GK rats, displayed histopathological islet changes. The general shape of islets became irregular, and immunoreactions of β-cells against anti-insulin appeared diffusely weakened. Electron microscopy revealed that the number of so-called β-granules decreased and the number of immature granules increased. The Golgi apparatus of β-cells was developed and the cisternae of rough endoplasmic reticulum were dilated, indicating hyperfunction of the cells. This study suggested that insulin deficiency in GK rats is not caused by simple dysfunction and/or degeneration of β-cells but rather by more complicated events within cells [50]. GK rat is one of the best characterized animal models used for studying the relation of changes in beta cell mass and occurrence of type 2 diabetes and diabetic complications (particularly diabetic nephropathy). However, only very few studies on drug testing using this model have been reported in the literature [51].

3) LEW.1WR1 rats:
Mordes, J.P *et al* have reported a novel rat model of autoimmune diabetes that arose in a major histocompatibility complex congenic LEW rat. Spontaneous diabetes in LEW.1WR1 rats (RT1$^{u/u/a}$) occurs with a cumulative frequency of ~2% at a median age of 59 days. The disease is characterized by hyperglycemia, glycosuria, ketonuria, and polyuria. The study explored that the islets of acutely diabetic rats are devoid of β-cells, whereas α- and δ-cell populations are spared. The peripheral lymphoid phenotype is normal, including the fraction of ART2$^+$ regulatory T-cells. The LEW.1WR1 rat is also susceptible to collagen-induced arthritis but is free of spontaneous thyroiditis. The LEW.1WR1 rat provides a new model for studying autoimmune diabetes and arthritis in an animal with a genetic predisposition to both disorders that can be amplified by environmental perturbation [52].

4) NONcNZO10 mouse:
The NZO strain is a polygenic model of obesity and diabetes obtained by selective inbreeding over several generations with the parents selected for their agouti coat color. It exhibits a polygenic syndrome of hyperphagia, obesity, mild hyperglycaemia, hyperinsulinaemia, impaired glucose tolerance and insulin resistance. Body weight rises rapidly during first 2 months of life due to hyperphagia. Hyperleptinaemia and leptin resistance which may account for hyperphagia, have been reported in NZO mice, a mouse model of the metabolic syndrome [53]. Obesity in NZO mice develops independent of the dietary sucrose or fat content, and of the fat quality. However, the dietary fat content accelerates the onset of diabetes without enhancing adiposity [54]. Reduced glycogen synthase activity in liver has been considered as a primary early defect in causation of diabetes [55].

5) C57BL/6J mice:
Type-2 diabetic model by simply feeding high fat feed to non obese, non diabetic C57BL/6J mouse strain was initially developed in Japan and is now available at Jackson laboratory, Bar Harbor. It is characterized by marked obesity, hyperinsulinaemia, insulin resistance and glucose intolerance [56]. In addition, they exhibit marked fasting as well as basal hyperglycaemia in contrast to normal basal glucose level seen in C57BL/6J (ob/ob) mice. C57BL/6J (B6) mice develop severe obesity and diabetes if weaned onto high-fat diets, whereas A/J mice tend to be obesity and diabetes-resistant. B6 mouse the severity of diabetes is a direct function of obesity and diabetes is completely reversible by reducing dietary fat [57]. Further, its usefulness for drug testing has been reported in the literature as these mice treated with orally active inhibitor of dipeptidyl peptidase-IV (LAF237) are shown to have normalized glucose tolerance in association with augmented insulin secretion [58].

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6) Kuo Kundo mice:

Kuo Kondo(KK) mice is polygenic model of obesity and type-2 diabetes produced by selective inbreeding for the large body size in Japan, also named as Japanese KK mouse. These animals are hyperphagic, hyperinsulinaemic, insulin resistant and show moderate obesity by 2 months of age, which attains maximum at 4-5 months. Insulin resistance precedes the onset of obesity [59]. The KK mice develop chemical diabetes preceded by a stage of prediabetes and also demonstrate renal, retinal and neurologic complications similar to those seen in human diabetes. Reddi *et al* have reported that the KK mouse serves as an ideal genetic animal for the study of non-insulin-dependent diabetes mellitus and its complications for rational prevention and therapy [60]. The increase in pancreatic insulin content is associated with increase in number and size of pancreatic islets but histologically degranulation of beta cells and hypertrophy of islets are found. There is selective failure of insulin to suppress gluconeogenic pathway, while exerting its inductive effect on glycolysis and lipogenesis as seen in hepatic insulin resistance of *db/db* mouse [61].

7) Tsumara Suzuki Obese Diabetes mice:

By selective breeding of obese male mice of ddY strain, Tsumara and Suzuki described the two inbred strains, one with obesity with increase in urinary glucose named TSOD (Tsumara Suzuki Obese Diabetes) and other without them (TSNO, Tsumara Suzuki Non Obese). TSOD mouse is of polygenic origin and characterized by polydipsia and polyuria at about 2 months old only in male mice followed by hyperglycaemia and hyperinsulinaemia [62]. The TSOD mouse has been established as an inbred strain with spontaneous development of diabetes mellitus as the first clinical signs of diabetes. Following these symptoms obesity gradually develops until about 12 months old. In histopathological examination of the pancreas, severe hypertrophy of pancreatic islets was observed due to proliferation and swelling of B cells. It has been shown that the TSOD mouse similar to NIDDM in humans, the TSOD mouse should be a useful model for the pathogenic study of diabetic complications, especially of peripheral neuropathy [63].

8) db/db mice:

The db/db (diabetic) mouse (now relabeled as lepr^{db}) is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KsJ strain originating from Bar Harbor, Maine .The mutation in this diabetic animal was traced to db gene, which encodes for the leptin receptors. These mice are spontaneously hyperphagic insulin over secretors becoming obese, hyperglycaemic, hyperinsulinaemic and insulin resistant within first month of age and develop hypoinsulinaemia, hyperglycaemia later with a peak between 3-4 months of age [64]. Animals then exhibit ketosis, progressive body weight loss and do not survive longer than 8-10 months [45]. db/db mice have been commonly and extensively used for the investigation of type-2 diabetes/diabetic dyslipidaemia and for screening of agents such as insulin mimetic and insulin sensitizers [65].

It is reported that the platelet function and coagulation not similar in db/db and ob/ob mice. Do not demonstrate a hypercoagulable state similar to humans with type-2 diabetes [66].

9) Obese rhesus monkey (Macaca mullata):

Obese rhesus monkey, an excellent non rodent model develops obesity, hyperinsulinaemia and insulin resistance when maintained on *ad libitum* laboratory diet, which gradually progresses to necrosis of beta cells, severe fall in insulin levels and overt hyperglycaemia over a period of several years. Unlike conventional rodent models, the final secretion loss is interestingly associated with deposition of amylin/amyloid in beta cells and the development of diabetic complications similar to human type-2 diabetes. Pioglitazone has been demonstrated to improve insulin resistance in obese rhesus monkeys [67, 68].

IV- Virus induced diabetic animal model-

There is a consensus among epidemiologists that the worldwide incidence rate of type-1 diabetes has been raising in recent decades. The cause of this rise is unknown, but epidemiological studies suggest the involvement of environmental factors, viral infections in particular. New evidence from animal models supports the hypothesis that viruses induce disease via mechanisms linked with innate immune up regulation. In the Bio Breeding Diabetes Resistant rat, infection with a parvovirus induces islet destruction via up regulation of the toll-like receptor 9 (TLR9) signaling pathway [69]. Viruses produce diabetes mellitus by destroying and infecting pancreatic beta cells. A less infecting or cytologic variant produces a comparable damage by eliciting immune auto reactivity to the β-cells.

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Various human viruses used for inducing diabetes include RNA picornoviruses, Coxackie B4, encephalomylocarditis (EMC-D and M variants), Mengo-2T, reovirus, and lymphocytic choriomeningitis [70, 71]. Data from retrospective and prospective epidemiological studies strongly suggests that enteroviruses, such as coxsackie virus B4 (CV-B4), may be associated with the development of T-1D. It has also been shown that enterovirus infections are significantly more prevalent in at-risk individuals such as the siblings of diabetic patients, when they develop anti-β-cell autoantibodies or T-1D, and in recently diagnosed diabetic patients, compared with control subjects. The isolation of CV-B4 from the pancreas of diabetic patients supports the hypothesis of a relationship between the virus and the disease [72].

Furthermore, studies performed in vitro and in vivo in animal models have increased our knowledge of the role of CV-B4 in T1D by helping to clarify the pathogenic mechanisms of the infection that can lead to β-cell destruction, including direct virus-induced β-cell lysis, molecular mimicry, bystander activation and viral persistence. The role of enteroviruses as the sole agents in T-1D, and a causal link between these agents and T-1D, have not yet been established, although arguments that support such a role for these viruses in the pathogenesis of the disease cannot be ignored [73].

V- Oral glucose loading animal model-

This method is often referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is transiently increased with no damage to the pancreas. In the clinical setting, it is known as Oral glucose tolerance test has been widely used for the diagnosis of impaired glucose tolerance, diabetes mellitus and gestational diabetes. Simultaneous measurements of plasma glucose and insulin (or more rarely C-peptide) levels allow to derive indices of insulin secretion and insulin sensitivity that are helpful for the understanding of disturbances in glucose metabolism and, especially, for the prediction of progression from normal glucose tolerance to impaired glucose tolerance or type-2 diabetes. Certain indices, quite simple, may be used in clinical practice ("insulinogenic index" to assess early insulin secretion, Matsuda index to assess insulin sensitivity) while others, more complex (and most often based on modelling procedures), are essentially used in research. The oral disposition index, a recently introduced marker that integrates insulin secretion and insulin sensitivity, raises increasing interest, more particularly for the prediction of type-2 diabetes [74].

Another study has reported highest possible reduction of causes of individual variability, at determination of diagnostic value of different intravenous and oral glucose loads and at comparison of diagnostic value of iv GTT and oral GTT after the same load. The authors first performed iv GTT with 1 g glucose per kg body mass and oral GTT with 1, 2, 4 g/kg in normal (non anaesthesized) adult (F 6-7) inbred rats and mice and then repeated these tests with the same loads and the same rats and mice with different extent of B-Cell lesion after i.v. alloxan injection. Results showed that for unmasking of latent ("chemical") diabetes a higher load (4 g/kg orally) was necessary and that the assimilation constant of iv GTT was diabetic earlier than the criteria of oral GTT after equal oral load (1 g/kg) and that for the diagnosis of early stages of diabetes not only absolute values of GTT curves but also their form are important [75].

VI- Insulin Antibodies-induced diabetes-

Bovine insulin along with CFA to guinea pigs produces anti-insulin antibodies. Intravenous injection of 0.25-1.0 ml guinea pig anti-serum to rats induces a dose dependent increase in blood glucose levels up to 300 mg/dl. This effect is due to neutralization of endogenous insulin by insulin antibodies. It persists as long as the antibodies are capable of reacting with insulin remaining in the circulation. Large doses and prolonged administration are accompanied by ketonemia, ketonuria, glycosuria and acidosis [76].

VII- *In-vitro* models for diabetes-

The need for alternative strategies for the prevention and treatment of diabetes is growing rapidly as type-2 diabetes is reaching epidemic status in our society. This need was the basis for the creation of this study, as it was necessary to start looking towards medicinal plants as potential antidiabetic treatment and no comprehensive *in vitro* model existed. Techniques for studying hypoglycaemic activity *in-vivo* employ animals with normoglycaemia or induced hyperglycaemia, as well as diabetic humans. *In vivo* bioassays are essential to prove the value of new hypoglycaemic agents, however, animal tests reveal relatively little about the specific mechanisms of action of the compound, and it is evident that there are a great many mechanisms by which blood glucose levels may be reduced [77].

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The lack of perfect models for type-2 diabetes, coupled with financial restrictions on obtaining and maintaining animals, and social restrictions on extensive use of animals in experimentation, indicate that a more practical approach would involve a series of *in vitro* prescreens before testing a potential new hypoglycaemic agent in animals. Many *in vitro* techniques have been developed to elucidate the varied mechanisms of action of hypoglycaemic agents discovered by *in vivo* bioassays. Three aspects of the hypoglycaemic response are commonly studied *in vitro*: insulin release from the pancreatic islets, peripheral insulin binding and glucose uptake, and effects on hepatic enzymes [78-82].

Table-1 The doses of various chemical diabetogens in different species

	Chemicals	Species	Dose (s) (in mg/kg)	References
➢	**Alloxan**	Rat	40-200 (iv or ip)*	7,24,53
		Mice	50-200 (iv or ip)	7,53
		Rabbit	100-150 (iv)	9,53
		Dog	50-75 (iv)	23,53
➢	**Streptozotocin**	Rat	35-65 (iv or ip)	8,53
		Mice	100-200 (iv or ip)	27,53
		Hamster	50 (ip)	8
		Dog	20-30 (iv)	4,6,7
		Pig	100-150 (iv)	10,53
		Primates	50-150 (iv)	11,53

* iv (intravenous)
*ip (intraperitoneal)

Table-2 Advantages and disadvantages of different categories of diabetic animal models

Model Category	Advantages	Disadvantages
➢ **Chemical induced diabetic animals**	• Selective loss of pancreatic beta cells (Alloxan/STZ) leaving other pancreatic alpha and delta cells intact. • Residual insulin secretion makes the animals live long without insulin treatment. • Ketosis and resulting mortality is relatively less. • Comparatively cheaper, easier to develop and maintain.	• Hyperglycemia develops primarily by direct cytotoxic action on the beta cells and insulin deficiency rather than consequence of insulin resistance. • Variability of results on development of hyperglycemia is perhaps high. • Diabetes induced by chemicals is mostly less stable and at times reversible because of the spontaneous regeneration of beta cells. Hence, care must be taken to assess the pancreatic beta cell function during long-term experiments. • Chemical produce toxic actions on other body organs as well besides its cytotoxic action on beta cell.
➢ **Surgical induced diabetic animals**	• Avoids cytotoxic effects of chemical diabetogens on other body organs. • Resembles human type-2 diabetes due to reduced islet beta cell mass.	• Involvement of cumbersome technical and post operative procedures. • Occurrence of some other digestive problems (as a result of part of excision of exocrine portion (deficiency of amylase enzyme). • Mortality is comparatively higher. • Dissection of alpha islets (glucagon secreting cells) too along with beta cells leading to problems in counter regulatory response to hypoglycemia. • The total resection of the pancreas in rat is very difficult to achieve and the development and severity of the diabetic state appear to be strain specific.
➢ **Genetically induced diabetic animals**	• Development of type-2 diabetes is of spontaneous origin involving genetic factors and the animals develop characteristic features resembling human type--2 diabetes. • Mostly of inbred animal models in which the genetic background is homogeneous and environmental factors can be controlled, allow genetic dissection of this multifactorial disease easy. • Variability of results perhaps minimum and require small sample size. • Effect of single gene or mutation on diabetes can be investigated *in vivo*. • Dissection of complex genetics of type-2 diabetes becomes easier.	• Expensive for regular screening method. • Highly sophisticated and costly procedure for the production and maintenance. • Limited availability and expensive for the diabetes study. • Mortality due to ketosis problem is high in case of animals with brittle pancreas disease easy (db/db, ZDF rat *P. obesus*, *etc*.) and require insulin treatment in later stage for survival. • Highly inbred, homogenous and mostly monogenic inheritance and development diabetes is highly genetically determined unlike heterogeneity seen in humans.

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CONCLUSION

In this review, we discussed a number of experimental animal models used in diabetes research. It is important to emphasize that diverse experimental animal models are essential for developing new anti-diabetic agents and for fully investigating promising agents before human clinical trials. It is expected that more therapeutic alternatives will become available with future advances in diabetes research.

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