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RECENT ADVANCES IN STUDY OF EXPERIMENTAL MODELS AVAILABLE FOR PHARMACOLOGICAL SCREENING OF ANTIDIABETICS

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ABSTRACT

Diabetes is a chronic disease. It might be a global morbidity. As a result, this condition poses a significant health risk. The quest for chemicals with the best qualities for treating illnesses is continuously ongoing. Experimental models are used in it. The goal of this study is to put all of the reported models together, highlighting their benefits and drawbacks, as well as the precautions that each model or approach requires. Diabetes comes in two forms. Type-I diabetes necessitated the use of insulin, but type-II diabetes can be managed in a variety of ways. Diabetes is caused by hyperglycemia, which is a leading cause of the disease. Hyperglycemia has a part in the development of diabetes as well. A rise in blood sugar level has a negative impact on the important organs. Diabetes impairs the function of the heart, kidneys, and eyes. This review article aims to bring together various animal models for antidiabetic activity screening, such as alloxan-induced and streptozotacin-induced diabetes in rats, as well as alternative animal models for antidiabetic activity screening, such as in vitro alpha amylase inhibiting assays, glucosidase inhibitory activity etc. as well as their benefits and drawbacks.

KEYWORDS: Hyperglycemia, animal model, alloxan induced, streptozotacin, diabetes mellitus, alpha amylase, glucosidase inhibitory activity.

INTRODUCTION

Diabetes mellitus is a carbohydrate, lipid, and protein metabolic disease. Glucose cannot be used in the body due to a lack of insulin release from the pancreas, resulting in diabetes. Diabetes is divided into two categories. The two types of diabetes mellitus are IDDM (insulin-dependent diabetes mellitus) and NIDDM (non-insulin-dependent diabetes mellitus). Diabetes mellitus affects 10% of the population, with 90% of those suffering from type 2 diabetes.[1] Diabetes mellitus is a carbohydrate, lipid, and protein metabolic disease. Glucose cannot be used in the body due to a lack of insulin release from the pancreas, resulting in diabetes. Hyperglycemia, hypercholesterolemia, and hypertriglyceridemia are all symptoms of type 2 diabetes. It is caused by insulin secretion abnormalities, impaired tissue sensitivity to insulin (insulin resistance), or a combination of both. It is a dangerous endocrine condition characterised by poor metabolic management, which raises the risk of cardiovascular illnesses such as atherosclerosis, renal failure, blindness, and diabetes cataract.[2]

The use of animal models to study diabetes mellitus is extremely helpful in understanding many elements of the disease's pathophysiology and in developing novel medicines and cures. Animal models are commonly employed and beneficial in biomedical research because they promise fresh insights into human diabetes. Rodents are used in the majority of animal models due to their small size, ease of availability, and cost. Chemical, surgical, and genetic modifications are among the animal models employed.[3]

The models are stated as follows

A. Animal models

I. Chemically induced diabetes Model
II. Surgically induced diabetes Model
III. Genetically induced diabetes Model
IV. Virus induced diabetes Model
V. Oral glucose loading model
VI. Insulin antibodies induced model

B. Alternatives to animal models

i. α-amylase inhibitory assay in vitro

II. α- glucosidase inhibitory assay in vitro

A. Animal models for Type– I and Type- II Diabetes

I. Chemical induced diabetes.

Chemicals are used to cause hyperglycaemia in animals. Diabetes causing agents are a type of chemical that may be divided into three types. Some of these chemical agents produce particular damage to -cells in the pancreas of animals or cause transient insulin production suppression, and these diabetogenic drugs reduce insulin's metabolic effectiveness in target tissue. The following are some texts that describe how these agents cause diabetes.

1. STZ (streptozotacins) induced diabetes

It's made up of glucosamine and nitrosourea. It has been through clinical trials since 1967. STZ can cause diabetes when given as a single injection or as a series of low-dose injections. STZ is a commonly used medication to induce diabetes in rats.[4]

In adult wistar rats, a dose of 60mg/kg STZ is injected intravenously, causing pancreatic swell and degeneration of islet-cells of Langerhans, as well as the ability to produce diabetes mellitus in 2-4 days. After 3 days of STZ treatment, -cells degenerate, and diabetes is generated in all animals. Nicotinamide-adenine dinucleotide (NAD) in pancreatic islet -cells induces histopathological changes in -cells, which most likely mediates diabetes development.[5]

The goal of the study was to look into the biochemical and histomorphological alterations caused by STZ-induced diabetes in rabbits.[6]

Insulin-mediated glucose metabolism in streptozotocin-treated diabetic pigs was studied to see if STZ diabetic pigs may be a good model for insulin-resistant type 2 diabetes.

That protects against the cytotoxic effects of STZ by scavenging free radicals and produces very The gradual infusion of STZ (130 mg/kg) in pigs on a low fat diet generates metabolic anomalies of type 2 diabetes mellitus as well as sensitivity to oral metformin treatment, according to this study.

The metabolic cause of insulin resistance in STZ diabetic pigs is most likely related to hyperglycemia and hyperlipidaemia.[7]

Streptozotacin is toxic to pancreatic beta cells, which are utilised to simulate Type I diabetes in a variety of Species, including nonhuman trimates. The study also involved inducing STZ in vervet monkeys (Chlorocebus aethiops) through intravenous injection of 45 mg/kg STZ (n=8, STZ-45) and 55 mg/kg STZ (n=12, STZ=55), with 10 monkeys receiving saline as a control group. Exogenous insulin needs increased quickly for four weeks, according to the findings. By maintaining the STZ-45 insulin dose while increasing the STZ-55 dose over the course of 16 weeks. In both groups, glucose tolerance tests and argenine-stimulated insulin production revealed an 80-90 percent loss in pancreatic-cell activity. STZ monkeys' body weight was likewise lowered, with only STZ-45 returning to baseline after 16 weeks. Glucose tolerance testing and argenine-stimulated insulin secretion confirmed 80-90% reduction in pancreatic β-cells function in both groups Body weight of STZ monkeys was also reduced with return to base line only in STZ-45 in 16 weeks. It is also found that increased blood urea nitrogen and creatinine levels were observed the STZ-55 group. It is also found that alkaline phosphate also increased with STZ-55 ($p < 0.05$ versus control) whereas STZ-45 alkaline phosphatase elevation was resolved by the end of study. This model concluded that diabetes mellitus can be induced and maintained in vervets monkey with a single dose of STZ. STZ (45 mg/kg) which significantly improved the toxicity profile without changing the efficacy in inducing diabetes mellitus. Finally, sufficient time following induction in recommended to resolve transient renal, hepatic and hematologic parameter.[8]

A single high dosage intraperitoneal injection of 100mg/kg body weight of STZ was used to induce severe insulin dependent diabetes mellitus (IDDM) in the musk (suncus murinus, insectivora). It was found that this is a one-of-a-kind animal that defined fatty liver and hyperlipidemia, and that it might be beneficial for investigating IDDM lipid metabolism.[9] According to the literature, doses and procedures for inducing diabetes in cattle utilising STZ should be addressed in the future.[10]

a) Streptozotocin induced diabetes Neonatal rat model. (in-STZ)

This model includes multiple stages of type 2 diabetes, such as glucose intolerance disturbances, mild, moderate, and severe glycemia.[11] In general, a single dosage of 100 mg/kg i.p. of steptozotocin to a one-day-old pup and 120 mg/kg i.p. to two, three, or five-day-old pups produces diabetes. The beta cells in n- STZ rats have characteristics that are similar to those reported in individuals with type 2 diabetes mellitus. As a result, the n-STZ model is one of the best models for type II diabetes.[12]

b) Nicotinamide- streptozotocin (NA-STZ) induced diabetic model

STZ is a streptomyces chromogenes-derived antibiotic that is structurally a glucosamine derivative of nitrosourea. Its direct cytotoxic impact on pancreatic beta cells causes blood sugar levels to rise (hyperglycemia). STZ's cytotoxic action in adult rats has resulted in an unique experimental diabetes state that appears to be more similar to NIDDM than other known animal models. The addition of NA was advantageous because it protects the intracellular pool of NAD by acting as a precursor of NAD or by reducing the activity of poly

(ADP ribose) synthetase, a NAD devouring enzyme induced by STZ. The dose of 230 mg/kg given intraperitonally 15 minutes before administration of STZ (65 mg/kg I.v) yielded moderate and stable non fasting hyperglycemia (155+3 vs 121+3 mg/dl in controls, P0.05) and 40% preservation of pancreatic insulin stores in maximum animals among the various dosages of nicotiamide tested in 3 month old wistar rats (100-350 mg/kg) body wt.[13] A single intraperitoneal injection of STZ (60 mg/kg) and NAD (120 mg/kg) into rats produced NIDDM. NAD is an antioxidant modest damage to type-II diabetes-producing pancreatic beta cells. As a result, this model is viewed as a useful tool for testing insulinotropic drugs in the treatment of type 2 diabetes.[14]

c) Sucrose- challenged streptozotocin induced diabetic rat model (STZ-S)

Male Albino rats are usually used in animal investigations as this model. Sucrose-loaded model is another name for it. The rats utilised were Charles faster/ wistar strain rats with an average body weight of 160+20 g. The STZ solution is produced by dissolving it in a PH 4.5 citrate buffer of 100mM. Overnight fasting rats (60mg/kg) were given the needed or estimated quantity of fresh solution intraperitoneally. After that, glucostrips was used to assess the blood glucose level after **48 hours.** Diabetic animals were defined as those with blood glucose levels between 144 and 270 mg/d1 (8-15mM). After 30 minutes, a sucrose load of 2.5 kg/body weight was administered. Glucostrips were used to assess blood glucose levels at 30, 60, 90, 120, 180, 240, 300, and 24 hours. Non-responders were defined as animals who had no diabetes 24 hours after therapy. During the experiment, the cages were denied food but not water.[15]

d) Low dose STZ with high fat diet fed rat model

This model is suitable for human type-II diabetes because it reflects the disease's natural history and metabolic features. It's also useful for pharmacological research. The rats are given a high-energy meal containing 20% glucose and 10% fat, as well as a single injection of STZ (30 mg/kg b.w.). The changes in body weights are reported after four weeks. Standard procedures are used to determine the levels of glucose, TG, TC, and LDL in the blood. According to the findings, a combination of low dosage STZ with a high-energy diet can successfully cause type 2 diabetes by modifying or modulating gene expression in important metabolic organs.[16.17]

2. Alloxan induced diabetes

It really is a uric acid derivative. At neutral pH, it is similarly very unstable in water, but it is stable at pH 3. With its reduction result, dialuric acid, it forms reactive oxygen species in a cylic redox process. Dialuric acid autoxidation produces superoxide radicals, hydrogen peroxide, and hydroxy radicals. These hydroxy radicals are responsible for the demise of cells with a limited antioxidative defence capability, resulting in alloxan diabetes that is insulin dependent.[18] The dose of alloxan varies by animal species, such as rats (40-200 mg/kg) i.v. or i.p., and mice (50-200 mg/kg) i.v. or i.p.[19] Alloxan, in general, induces diabetes in three stages. The dose of alloxan varies by animal species, such as rats (40-200 mg/kg) i.v. or i.p., and mice (50-200 mg/kg) i.v. or i.p.[19] Alloxan, in general, induces diabetes in three stages. Early hyperglycemia of short duration (approximately 1-4 hours) caused by insulin release stoppage has direct glycogenolytic effects on the liver in stage I. Stage 2 hyperglycemia can persist up to 48 hours and can lead to convulsions and death. In animals with fully established alloxan diabetes, stage III chronic diabetic phase occurs owing to a lack of insulin histologically only a few β-cell if any are detectable in animals with fully developed alloxan diabetes. Exogenous insulin readily restored normal blood glucose levels.[20]

Diabetes mellitus was experimentally generated in New Zealand white male rabbits by administering four doses of alloxan intraporitoneally at weekly intervals after a 12-hour fast. In diabetic rabbits with untreated diabetes, adverse effects were observed in the pancreas, kidneys, lungs, heart, and brain. The histological changes affect practically all of the body's organs. Mild alterations in the gastrointestinal system were discovered, together with yeast growth in the stomach, indicating an increase in the vulnerability of the gastric mucosa to yeast cell proliferation.[21]

In another research, alloxan and magnesium had an effect on plasma free fatty acid in rats. After administering Alloxan 120mg/kg intraperitoneally to 28 rats for 72 hours, plasma glucose measurements revealed diabetes induction. In comparison to the control group, plasma free fatty acids exhibited a considerable rise (751.25mM) (286.68mm). The magnesium content in red blood cells was also shown to be significantly lower in diabetic rats, dropping from 7.18 mg/dl to 4.89 mg/dl. In diabetic patients, the study found an inverse connection between plasma free fatty acids and red blood cell magnesium. Analysis of red blood cell magnesium after induction of diabetes might give useful information for diabetes treatment.[22]

Table 1: Shows the dosages of several chemical diabetogens in various species.

Chemicals	Species	Dose(in mg/kg)	References
Alloxan	Rat	40 -200 iv. ip	6
	Mice	50-200(iv.ip)	6
	Rabbit	100-150(iv)	8
	Dog	50-75(iv)	21
Streptoxotocin	Rat	35-65(iv.ip)	7
	Mice	100-200(iv. ip)	23
	Hamster	50(ip)	7
	Dog	20-30(iv)	6
	Pig	100-150(iv)	9
	Primates	50-150(iv)	10

II. Surgically induced diabetes

In animals, this approach involves full or partial pancreatctomy. It is used to induce type I or type II diabetes, depending on the situation. The diabetic dog model discovered by Oskar Minkowski by surgical total pancreatectomy was formerly thought to be the first animal model of diabetes, however it is currently only used infrequently for research.[24] This paradigm has been utilised by a small number of researchers to investigate the effects of natural products on animal species such as rats, pigs, dogs, and primates.[25,26] On animals, particularly non-rodents, partial pancreatectomy and/or combination procedures are commonly used.

III. Genetically induced diabetic animal model

Type 2 diabetic animals are seen in animals that have one or more genetic mutations that are passed down from generation to generation. ex-db/db mice. Because of interactions between environmental factors and various gene abnormalities, individuals are more likely to develop type 2 diabetes. As a result, as compared to monogenic animals, polygenic animals are more resemble human conditions.[27,28]

IV. Virus induced diabetic model

The concept that viruses induce disease through processes connected to innate immune upregulation is associated with new evidence from animal models. Infection with a parvovirus causes islet destruction in the Bio breeding diabetes resistant rat via upregulating the toll-like receptor 9 (TLR9) signalling pathway.[29] Viruses generally damage and infect pancreatic beta cells, resulting in diabetes. By evoking immune auto reactivity to the cells, the cytological variant causes equivalent harm. RNA picornaviruses, coxackie B4, encephalomylocarditis (EMC-D and M Variants), mengo-27, reovirus, and lymphocytic chromeningitis are examples of diabetes-inducing viruses.[30,31] Entroviruses like coxsackie virus B4 (CV-B4) are substantially linked to the development of T-I Diabetes, according to prospective epidemiological research. The isolation of CV-B4 in a diabetic patient's pancreas supports the theory of a link between the virus and the condition.[32] The involvement of enteroviruses as sole agents in T-I D and the existence of a causal relationship between these agents and T-I D has yet to be demonstrated, despite the fact that there are reasons that indicate such a role for these viruses in the disease's pathogenesis.[33]

V. Oral Glucose loading animal model

For the diagnosis of impaired glucose tolerance, diabetes mellitus, and gestational diabetes, this model is commonly utilised. It causes diabetes by not causing damage to the pancreas. The oral disposition index, a newly established metric that combines insulin secretion and insulin sensitivity, is gaining popularity, particularly for type-II diabetes prediction.[34]

VI. Insulin Antibodies- induced diabetes

Anti-insulin antibodies are produced when guinea pigs are given bovine insulin and CFA. Injecting rats with 0.25-1.0 ml guinea pig anti- serum causes a dose-dependent rise in blood glucose levels up to 300 mg/dl. Insulin antibodies neutralise endogenous insulin, which is the mechanism behind this action. It lasts as long as the antibodies are capable to responding to the residual insulin in the bloodstream. Ketonemia, ketouria, glycosuria, and acidosis are common side effects of large dosages and extended therapy.[35]

B. Alternative to animal model

In vitro models for diabetes

Animals with norm glycemia and induced hyperglycaemia, as well as diabetic people, were used to evaluate hypoglycemic activity in vivo. Animal tests reveal relatively little about the specific mechanism of action of the compound, and it is clear that there are many mechanisms by which blood glucose levels can be reduced. In vivo bio assays are required to prove the nature of new hypoglycaemic agents; however, animal tests reveal relatively little about the specific mechanism of action of the compound.[36]

Due to a lack of a suitable model for type 2 diabetes, economical considerations in obtaining and maintaining animals, and social restrictions on the widespread use of animals in studies, there is no perfect model for type 2 diabetes. A more feasible method would comprise a succession of in vitro models before trying a potential novel hypoglycemic drug in animals, according to the authors. In vitro insulin release from pancreatic islets, peripheral insulin binding and glucose absorption, and

the action of hepatic enzymes are three elements of the hypoglycemic response that are widely studied.[37-41]

I. In vitro α-amylase inhibitor assay

The Bernfeld technique was used to test the inhibitory action of alpha amylase in vitro.[42] The test extract (0.02-4 l) was given 200 µl of amylase enzyme (Himedia RM 638, Mumbai) and 100 µl of 2 mm phosphate buffer to react with (pH, 6.9). After a 20-minute incubation period, 100 l of 1% starch solution was added. The controls, in which 200ul of enzyme was replaced by buffer, were carried out in the same way. 500l of dinitrosalicylic acid reagent was added to both the control and test after 5 minutes of incubation. For 5 minutes, they were immersed in boiling water. The absorbance of the sample was measured using a 540nm rising spectrophotometer, and the percent inhibition of the amylase enzyme was determined using the formula below.

% inhibition= (control - test) / control X 100

At the same time, appropriate reagent blank and inhibitor controls were carried out. All values were expressed as mean + std. Error of mean (SEM) (n=3).

II. α- glucosidase inhibitory activity

The enzyme inhibitory activity of alpha glucosidase was evaluated using the method reported by Shibano et al. (1997), with minor modifications (43). 50ul of 0.1 m phosphate buffer (pH 7.0) and 25l of 0.5 mm 4-nitrophenyl-D-glucopyranoside were used in the process (dissolved in 0.1 m phosphate buffer with pH of 7.0). 0.2 to 4 litres of oil and 25 litres of alpha glucosidase solution (a stock solution of 1 mg/ml in 0.01 m phosphate buffer with a pH of 7.0 was diluted to 0.1 unit/ml with the same buffer with a pH of 7.0 right before the assay). After that, the reaction mixture was incubated for 30 minutes at 370°C. The reaction was then stopped by adding 100L of 0.2 M-sod. Solution of carbonate. The quantity of P-nitrophenol released in the reaction mixture at 410 nm was used to monitor the enzymatic hydrolysis of the substrate. Individual blanks were made by adjusting the background absorbance and replacing the enzymes with buffer controls. The test extract was replaced with methanol in the same way. As a positive control, acarbose was employed. The mean + SEM (n=3) was used to express all of the results.

CONCLUSION

In this review, we examined a variety of animal models and alternatives to animal models that are utilized in diabetes research. Emphasis should be placed on discovering new anti-diabetic drugs as well as thoroughly researching promising compounds prior to human clinical trials. With future advancements in diabetes research, new therapy options are projected to become accessible.

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