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An Experimental Models (In-Vivo and In-Vitro) Used for the Study of Antidiabetic agents

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Abstract

Diabetes is divided into two main types, type 1 (insulin-dependent diabetes Mellitus) and type 2 (non-insulin dependent diabetes). About 90% worldwide diabetics have type 2 diabetes. They are different in vivo and in vitro methods for screening new diabetes drugs. Mainly in life models uses chemicals like streptozotocin, alloxan, etc. to induce diabetes in vitro methods directly demonstrate its effect on cells responsible for induction of diabetes in humans. In vitro techniques provide more accurate information and possible Mechanisms related to diabetes. Now the latest techniques of the day such as the induction of diabetes by viruses that have also been introduced proves to be a good tool in the evaluation of diabetes drugs. This review can prove it be a good tool for researchers who want to investigate the diabetes it provides a comprehensive resource on the diabetes model under one roof.

Keywords: *Diabetes, Investigate, Streptozotocin, Alloxan, Humans*

1. Introduction

Diabetes mellitus (DM) is a major public health problem affecting more than 400 million people worldwide. This metabolic disorder gradually leads to chronic microvascular, macrovascular and neuropathic life-threatening complications. DM is caused by deficiency insulin secretion, pancreatic β-cell damage or insulin resistance associated with not using insulin. A tendency to a sedentary lifestyle can be the main reason for the continuous increase in the number of patients with diabetes worldwide, which is expected to affect 366 million elderly people in 2030 population (> 65 years). It comes with various complications [1].

DM includes nephropathy, cardiovascular and renal complications, retinopathy, dietary disorders, etc. Type 1 DM and type 2 DM there are two types of DM. Type 1 DM is an autoimmune disease which affects pancreatic cells by reducing or weakening their production insulin, while type II DM is the result of pancreatic beta cell deficiency which interfere with a person's ability to use insulin [2]. Diabetes causes nerve damage or myocardial hypertrophy in the heart, which can cause diabetic cardiomyopathy, affects its development blood vessels in the retina, which can cause visual symptoms and sometimes blindness (diabetic retinopathy), causes scarring and fibrosis of kidney tissues, which can lead to chronic kidney disease(diabetic nephropathy), also affects the nervous system and diabetes beta neuropathy and which causes numbness, tingling and pain in the legs (diabetic foot), impairs sperm quality and destroys sexual dysfunctions, which causes varying degrees of male fertility (diabetic fertility damaged [3].

For determining the right therapy, the involved type of diabetes plays a key role and in 2018 American Diabetes Association (ADA) proposed the following classification: Type 1 diabetes mellitus (T1DM): due to autoimmune-cell destruction, usually leading to absolute insulin deficiency; Type 2 diabetes mellitus (T2DM): due to a progressive loss of-cell insulin secretion frequently on the background of insulin resistance. Gestational diabetes mellitus (GDM): diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt prior to gestation; Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young (MODY)), diseases of the exocrine pancreas (such as cystic

fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation) [4].

Experimental studies of diabetes in animal models and advanced in vitro techniques are necessary for knowledge and clear understanding of pathology and pathogenesis and find a new therapy. Therefore, animal models of diabetes are very useful in biomedical research because they provide new insights to human diabetes. Most of the available models are based on rodents due to small size, shorter generation intervals and economy Note. Several studied experimental diabetes methods that include chemical, surgical and genetic manipulations. It is also very important to choose an appropriate animal model screening for new chemical entities (NCEs) and other therapeutic agents' treatment of diabetes.

As the main goal this review aims to bring together all the different into live animal models and in vitro methods for diabetes research [5]. The prevalence of obesity has increased worldwide a rapid increase was observed in children, adolescents, and in adults, in some developing countries more than 50% in men.try. Increase in the number of diabetics in each region from 2017 and 2045 and the percentage increase was highest in geographic regions that include developing countries, namely Africa (16 million, 41 million; 156%), Middle East and North Africa (39 m, 82 m; 110 %, i.e. dense), South and East Asia (82 m, 151 m; 84%, left dense) and in South and Central America (26 m, 42 m; 62%) compared to Europe, North America and the West Pacific Ocean10 (see Tables 1–2).

About 79% of adults people with diabetes live in low- and middle-income households in countries. Hence the increase in diabetes in developing continues to cause huge medical and financial problems for economies infectious diseases are challenges. General analysis including various aspects of diabetes, especially syndromes infectious diseases and shortages and innovations in advance treatment, diagnosis and clinical management [6].

Screening Methods of In Vivo and in Vitro Methods

In Vivo Studies Diabetes Induction With Chemicals

Most jobs are offered in the Ethno meadow pharmacology from 1995 to 2007 used it model Streptozotocin (STZ, 69%) and alloxan (31%) are by far the most used drugs and this model was useful for exploring several aspects disease Both drugs have an antidiabetic effect if they are administered parenterally (intraperitoneally, intravenously or under the skin). Required dose these diabetes-causing agents depend animal type, method of management [7].

Streptozotocin Model of Diabetes Mellitus

Streptozotocin prevents the development of DNA bacterial cells and mammalian cells. It affects cytosine groups of bacteria that cause recurrence and damage DNA.10 Streptozotocin attack on the pancreas the cell is through the glucose transporter GLUT2 and causes DNA alkylation (Figure 1). STZ also convinces with actions ribosylation of polyadenosine diphosphate and release of nitric oxide. as a result of STZ function, pancreatic cells are destroyed as a result of necrosis.

Adult male Wistar rats should be housed downstairs under controlled laboratory conditions temp 25±3°C with 60±15% humidity and 12 h in darkness/light cycle Male Wistar rats (160-240 g) should be kept above standard chow diet and water ad libitum. Streptozotocin (60 mg/kg) administered as an intravenous injection. Initially, blood sugar increases 150-200 mb% within three hours after administration of streptozotocin.

The stage of hypoglycemia manifests itself four times increase in serum insulin levels and this phase is monitored with persistent hyperglycemia.12 Recently new an animal model of type 2 diabetes has been proposed combining STZ and NAD in adult rats (160-240 g). Rats were injected with NAD (230 mg/kg, ip) for 15 min. before STZ (65 mg/kg, iv) administration.On that basis, it showed a fair and definitive no fasting hyperglycemia without significant changes insulin level. NAD is an antioxidant that works Protective effect on the cytotoxic effect of STZ free radicals [8-10].

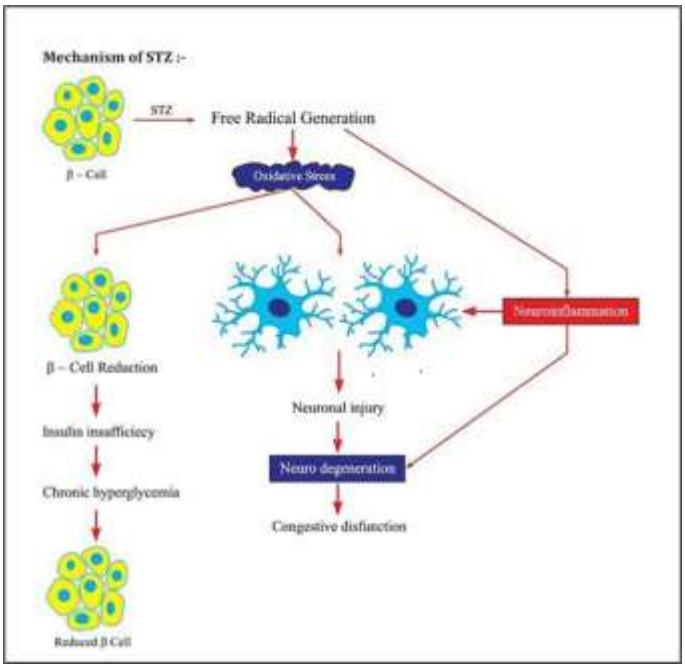

Figure 1: mechanism of action of streptozotocin

Alloxon Model of Diabetes Mellitus

Alloxan is also one of the chemicals used in induction in case of diabetes. It is a famous diabetogenic substance is widely used to ensure type 1 diabetes in animals. Alloxan is a urea derivative that causes selective necrosis of pancreatic islet β-cells (Figure 2). This urea derivative often used to induce diabetes in animals such as rabbits, rats, mice and dogs. In New Zealand white rabbits (2.5-4 kg), alloxan monohydrate (5 g/100 ml, pH 4.5) is infused. through the peripheral vein of the ear at a dose of 150 mg/kg at the time 10 min course. After these injections 70% of animals become hyperglycemic and uricosuric. Wistar or Sprague Dawley rats (150–200 g), Alloxan monohydrate is injected under the skin 100-175 mg/kg. Male dogs (15-20 g) are injected internally 60/mg/kg alloxan monohydrate intravenously. After that they get 1000 ml of 5% glucose solution with 10U of regular insulin iv for 1 week along with food ad libitium [11-12].

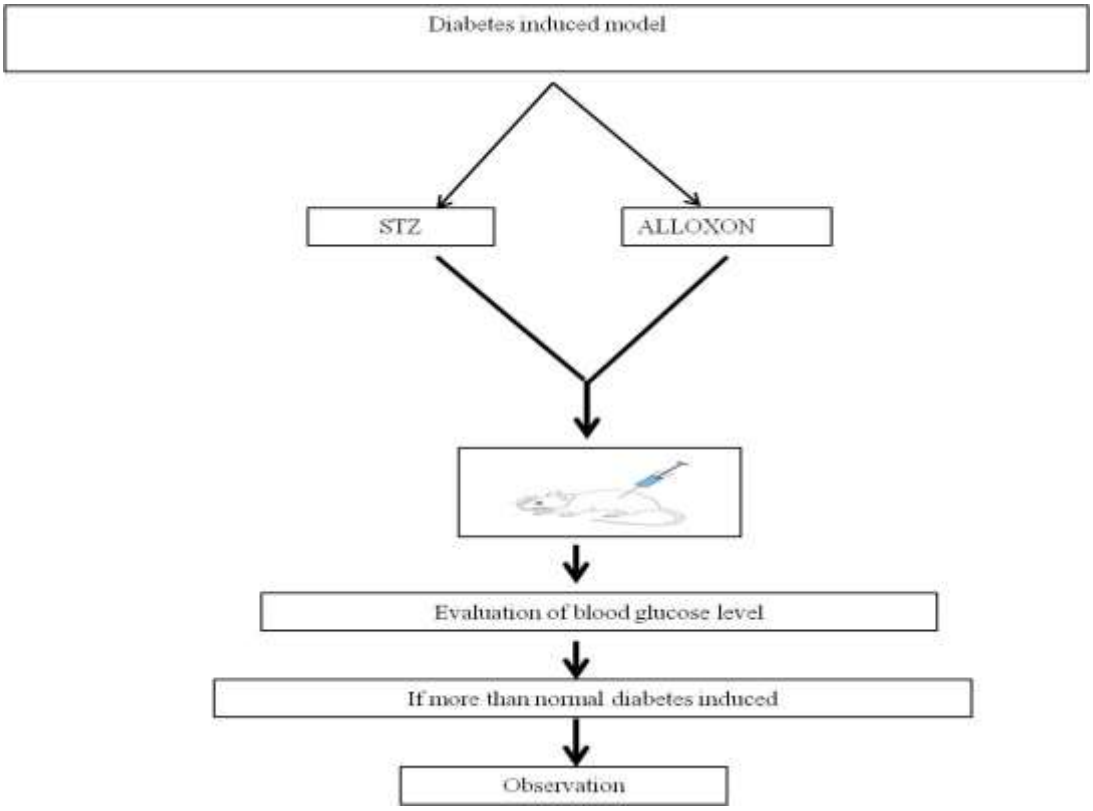

Figure 2: Pictorial representation of in vivo activity

Dithizone Induced Diabetes

Dithizone is an organosulfur compound that is commonly used Detection of diabetes in experimental animal models. Administration of dithizone to animals causes levels the amount of zinc, iron and potassium in the blood increases more than usual. Dithizone after death membranes form a complex with zinc, which causes the release of protons, thus ensures diabetogenicity. Injection of various chelating agents such as dithizone, 8-(p-toluenesulfonylamino) quinoline (8-TSQ) and 8-(benzenesulfanylamino)quinoline (8-BSQ) as a singlet i.v. dose 40-100 mg/kg for cats, rabbits, goldfish hamsters and mice cause type 2 diabetes. Dithizone injection produces a triphasic glycemic response in rabbits. Stages of initial hyperglycemia are observed after 2 hours, followed by a normoglycemic phase after 8 hours and seconds persistent persistent hyperglycemic phase after 24-72 hours. Histologically complete and partial degranulation beta cells are observed [13-14].

Monosodium Glutamate Induced Diabetes

Monosodium glutamate (MSG) increases glutamate levels concentration in plasma. MSG activates the release of insulin. Administration of MSG to mice causes obesity, which is increase in the level of insulin. It also increases blood circulation level of glucose, total cholesterol and triglycerides. Adult male Wistar rats are kept under control in laboratory conditions at a temperature of 25±3°C 60±15% humidity and 12 h dark/light cycle. Everything rats are fed ad libitum with standard rat chow pellets and provided drinking water purified by reverse osmosis (RO), with or without monotonic glutamate. There are eighty rats randomly into four groups to be monitored1, 3, 6 or 9 months, 20 rats in each group. Each group included control (n = 10) and MSG-treated (n = 10) rats. MSG-treated rats are supplemented commercially available with 99% pure food grade MSG into daily drinking water with a final daily dose of 2 mg/g body weight Food intake and body weight are recorded respectively every one or two weeks and rats of different groups were killed at 1, 3, 6, or 9 months of age After a 12-hour fast with intraperitoneal Nembutal injection Blood and pancreatic tissue were collected functional and morphological study [15-16].

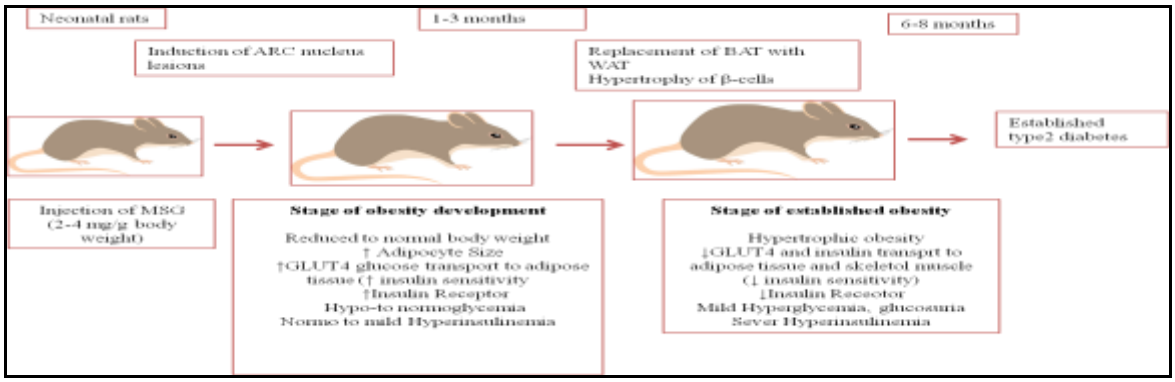

Figure 3: Monosodium Glutamate Induced Diabetes

Insulin Antibodies Induced Diabetes

Anti-insulin antibodies have an affinity and ability to bind insulin The mechanism of insulin deficiency can lead to greater postprandial hyperglycemia because antibody-bound insulin is not available to tissues, but prolonged postprandial hyperinsulinemia can lead to hyperglycemia [17-18].

Ferric Nitrilotriacetate Induction of Diabetes

In experimental animals, parenteral administration of a high daily dose of ferric nitrilotriacetate for 60 days causes diabetic symptoms such as hyperglycemia, glycosuria, ketonemia, and ketonuria [19].

Goldthioglucose Obese Diabetic Mouse Model

Goldthioglucose (GTG) is a diabetes-causing compound, causing obesity-induced type 2 diabetes. Experimental intraperitoneal administration of GTG the animal gradually develops obesity, hyperinsulinemia, and hyperglycemia. GTG is transported into the cell and causes necrotic lesions that are conscient increase in hyperphagia and obesity. GTC is also growing hepatic lipogenesis, body lipid content and triglycerides secretion [20-21]. Swiss albino mice of both sexes are fed commercial substances mouse meal optional. At six weeks of age, animals receive a single intraperitoneal injection 30-40 mg/kg goldthioglucose. Food is in take registered duration 2 weeks and body weight 3 months and compared to untreated controls [22-23].

Virus Induced Diabetes

Diabetes is mainly caused by viruses destroys and infects pancreatic beta cells. a lot human viruses have been used to cause diabetes, for example Mengo-2T, reovirus, Coxackie B4, RNA

picornaviruses, encephalomyocarditis (variants EMC-D and M) and lymphocytic choriomeningitis [24-25].

Coxsackie Viruses

Coxsackie viruses also cause diabetes in mice destroys pancreatic cells. Coxsackie B4 virus is strongly associated with insulin-dependent development diabetes in humans. Causes of diabetes Coxsackie virus infection causes bleed isolated islet antigen due to restimulation of autoreactive T cells [26-28].

Spontaneous Diabetic Obese Rodent Models

Ob/Ob Mouse

Leptin deficiency in ob/ob mouse strain due to mutation, the leptin gene causes severe insulin resistance. The strain ob/ob shows rapid weight gain, insulin resistance and hyperinsulinemia. In the ob/ob model hyperinsulinemia occurs at the age of 3-4 weeks at the same time simultaneously with insulin resistance and hyperphagia. The symptom of type 2 DM in ob/ob mice increases with age with a continuous decrease in plasma insulin levels [29-31].

DB/DB MOUSE

Mutation of the Db gene occurs impulsively C57BL/KsJ mice with leptin receptor-deficient. Pressure is originally derived from a chromosomal mutation number. Db/db mouse becomes hyperphagic, hyperinsulinemia and insulin resistance states 2 weeks old. Obesity occurs at the age of 3 years 4 weeks with the onset of hyperglycemia in age 4-8 weeks.36 The db/db mouse is used for research microvascular diabetic and renal complications [32-33].

Kuo Konda Mouse

The Kuo Kondo (KK) mouse is a model of obesity and Type 2DM. KK looks instinctively versatile hyperglycemia, hyperinsulinemia and obesity. At the age of 2 months, the KK mouse becomes ill due to obesity to insulin resistance, compensatory hyperinsulinemia and hyperphagic. Insulin resistance and hyperinsulinemia peaks at 5 o'clock months [34-35].

Zucker Diabetic Fatty (ZDF) Rat

The Zucker diabetic fatty (ZDF) rats are more insulin resistant, less obese, and quickly development to diabetes due to lack of adequate insulin secretion.42 The male ZDF rat develops fully diabetic conditions at 12 weeks. The serum insulin levels normally reach the peak at about 7 to 10 week [36-37].

Cohen Diabetic Rat

It is a genetic model resulting from diet-induced Type 2 DM model by introducing the rat on a synthetic 72% sucrose copper-poor diet for 2 months. The symptoms include insulin resistance, hyperinsulinemia, and non obesity. The Cohen diabetic rat expresses genetic receptiveness to a carbohydrate-rich diet which is a feature of Type 2 DM in human [38].

Surgical Models of Diabetic Mellitus

This technique completely removes the panacea cause diabetes. This model was mostly used in recent years in animal species such as rats, pigs and dogs and primates to study the effects of natural products. This technique has several disadvantages[39-40] including

(1) Good technical knowledge and suitable operating room atmosphere,

(2) Good hands in big cuts and high risk of animal infection,

(3) Appropriate postoperative administration of analgesia and antibiotics,

(4) Augmentation pancreatic enzymes in food to prevent malabsorption [41-42].

Genetic Models of Diabetes

Spontaneously Develop Diabetic Rat

These models allow the evaluation of antidiabetic effect of a natural product in an animal without the meddling of adverse effect induced by drugs like alloxan and Streptozotocin. An example for this is spontaneously diabetic Goto-Kakizaki rat, is a genetic lean model of type1 diabetes evolving from careful breeding over many generations of glucose-intolerant diabetic wistar rats.50Concerning type1 diabetes models, the mouse characteristically generates hyperglycemia between 12 and 30 weeks of age, whereas in BB rats it occurs around 12 weeks of age. One of great benefit of these mod els is that they can be used as model of atherosclerosis which generally represents the enduring impediment of diabetes mellitus [43-44].

Genetically Engineered Diabetic Mice

There is a significant advancement in the field in recent years, especially with the advent of transgenic mice. In this case, rodents may be evolved to over (transgenic) or under (knockout) - express proteins considering playing a key part in glucose metabolism. Presently there have been no protocols carried out concerning natural prod ucts and these models. Possibly, the high costs limit their study in complicated protocols which explore mechanisms of potential beneficial agents [45-47].

In-Vitro Methods

Assay of Amylase Inhibition

600 µl of (10,20,40,60,80,100µg/ml) test sample, 1.2 ml of starch in phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride are added. The reaction is initiated by adding 600 µl porcine pancreatic amylase and incubated at 37 ºC. From the above mixture 600 µl is taken and 300 µl of DNSA (1g of DNSA, 30g of sodium potassium tartarate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and is kept in a boiling water bath for 15 min [48]. The reaction mixture diluted with 2.7 ml of water and absorbance is read at 540 nm. For each concentration, blank tubes are prepared by replacing the enzyme solution with 600 µL in distilled water. Control, representing 100% enzyme activities are prepared in a similar manner, without test sample. The experiments are repeated thrice using the same protocol [49].The α-amylase inhibitory activity was calculated by using the formula

Abs 540 nm (Control)-

$$Inhibition\% = \frac{Abs\ 540nm(drug\ sample)}{Abs540nm(control)} \times 100$$

Inhibition of α glucosidase Activity

Enzyme solution is prepared by dissolving 0.5 mg α glycosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. The solution is further diluted in the ratio of 1:10 with phosphate buffer [50]. Sample solution should be prepared by dissolving 4 mg sample in 400 µl dimethyl sulfoxide (DMSO) and was referred as sample blank. Five concentrations 50, 100, 150, 200, and 250 µml are prepared. To each 5 µml of the sample solution and DMSO add P- nitro phenyl- α-D-glucopyranoside with phosphate buffer (ph7.0). The solutions are incubated at 37 $^{\circ}C$ for 15 min. After 15 min add Na_2CO_3 (1000µl) solution. Absorbance of the sample against sample blank is measured at 400nm using UV visible spectrophotometer [51].

The inhibition activity is calculated according to the formula-

$$\%Inhibition = \frac{Ec-(ET-EC)}{EC} \times 100$$

In-Vitro Studies on Insulin Secretion

Antidiabetic agents can influence many pathways of glucose metabolism such as insulin discharge, glucose uptake by target organs as well as nutrient absorption. Recently, in vitro studies were carried out considering incretins 58 and transcription factors such as peroxisome proliferators activated receptors· PPAR are targets of modern therapy. Insulin receptor, glucose transporters, on the other hand, has not been yet the target of anti diabetic therapy. There are few studies revealing the use of natural products has been published [52-54].

Studies Using Insulin-Secreting Cell Lines

Development in Bioengineering technologies have given various new methods to develop and create more suitable cultured cell lines to assist studies of mechanisms of both insulin secretion and cell dysfunction. The most extensively used insulin-secreting cell lines are beta-TC, RIN, HIT, MIN6 and INS-1 cells. These cell lines liberate mainly insulin, small amounts of glucagon and somatostatin. The actions of these cell lines never completely reduce primary cell physiology but, they are S527 tremendously precious tools for the study of molecular proceedings underlying cell function [55].

Studies Using Isolated Pancreatic Islet Cell Lines

The pathway which is responsible for diabetes can be studied with isolated pancreatic β-cells from either control or transference of these beta cells to appropriated culture medium [56]. It is a well-known phenomenon that insulin emission occurs when pancreatic cells make use of glucose to create adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The raise in cytoplasmic

ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage dependent Ca^{2+} channels [57]. This results in rise of intracellular Ca^{2+} level which initiates insulin secretion. In type 2 diabetes, pancreatic cells show uncharacteristic ion channel activity and an atypical pattern of insulin secretion [58]. These pathways can be depicted with isolated pancreatic cells from either control or diabetic rat or mouse that can be obtained by collagenase digestion technique, followed by adequate separation and transference to appropriated culture medium [59].

In-Vitro Studies on Glucose Uptake

The main key link between obesity and Type 2 diabetes is adipose tissue because it promotes the progress of lip toxicity, i.e. cell destruction because of prominent intracellular lipid concentrations and insulin resistance. The resistance of insulin either at the adipocytes or skeletal muscle levels causes hyperglycemia. However, adipocytes located on different sites of the body may have diverse biological or pathological effects. Insulin resistance pathways may be studied in cell lines of adipocytes such as marine 3T3-L1 cells and rat L6 muscle engineered to over-express GLUT4[60-61].

4. Conclusion

In this review an emphasis had been made to cover all in- vivo models and in- vitro techniques for the researcher who is seeking their research work in diabetes. In-vitro models had been described on the basis that animal's models have near about same characteristics features as human diabetes. Each model mentioned above are necessary tools for researching about endocrine physiology, metabolic changes and genetic changes involved in mechanism of occurrence of diabetes in human. More emphasis should be made on development of newer in vitro techniques for the evaluation and treatment of diabetes. In vitro methods used now days may be costly, but the result assessed through them confers the exact mechanism of diabetes occurrence. Still, more animal models and software based study should be developed for more advancement in diabetes research.

Abbrevations

STZ: Streptozotocin;
DNA: Deoxyribonucleic acid; Glut-2:

Glutamate 2 receptor;

NAD: Nicotinamide;

MSG: Monosodium glutamate;

GTC: Goldthioglucose;

EMC-D: Encephalomylocarditis;

KK: Kuo Kondo Mice;

ZDF: Zuker Diabetic fatty rats.

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Conflict of Interest

The authors declare no conflict of interest**.**

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Ethical approvals

This study does not involve experiments on animals or human subjects.

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