World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Review Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2024
Volume: 3; Issue: 6
Page: 307-323

A COGNIZANCE ON *IN-VITRO* AND *IN-VIVO* SCREENING TECHNIQUES FOR ANTIDIABETIC ACTIVITY

Sarojini Nayak[1]*, Durga Madhab Kar[2], N. Saroj Kumar Choudhury[3]

[1*]Department of Pharmacology, Kanak Manjari Institute of Pharmaceutical Sciences, Rourkela, Odisha, India.

[2]Department of Pharmacology, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan, Bhubaneswar, Odisha, India.

[3]Department of Pharmacy Wing, SCB Medical College Hospital, Cuttack, Odisha, India.

Article Received: 26 October 2024 | | ***Article Revised: 17 October 2024*** | | ***Article Accepted: 10 December 2024***

***Corresponding Author: Sarojini Nayak**
Petro Mohyla Black Sea National University.
DOI: https://doi.org/10.5281/zenodo.14576685

How to cite this Article: Sarojini Nayak, Durga Madhab Kar, N. Saroj Kumar Choudhury (2024). A COGNIZANCE ON *IN-VITRO* AND *IN-VIVO* SCREENING TECHNIQUES FOR ANTIDIABETIC ACTIVITY. World Journal of Pharmaceutical Science and Research, 3(6), 307-323. https://doi.org/10.5281/zenodo.14576685

ABSTRACT

Diabetes mellitus (DM) affects a large number of people worldwide and is a metabolic and endocrine issue. Human diet and lifestyle have undergone drastic changes lately, causing a range of diseases and chronic disorders, notably metabolic diseases such as obesity as a result. The absence of insulin characterizes this condition, which is linked to hyperglycemia. It remains a challenge to find unique drugs that treat the disorder with new properties. There is no cure for diabetes; however, if ignored, hyperglycemia can cause permanent damage to the blood vessels, neurons, and many other systems in the body, including the nervous system. The goal of the current review is to examine all published models and innovative approaches. This review mentions screening new antidiabetic drugs *in vivo*, *in vitro* and cell line technique. Streptozotocin and Alloxan are commonly used *in vivo* as chemicals, but apart from these chemicals, Dithizone, gold thioglucose, monosodium glutamate, and many more are specified. The artificial insemination technique often includes the α-amylase restriction measure and the α-glucosidase inhibition examination, which are frequently discussed in various papers. However, other tests, such as the insulin emission test, are also described. Cell line methods such as isolated pancreatic islet cell line, insulinoma cell line, and countless techniques are present in this article. Counter-diabetic action of different specialists in cell line models adds to giving a superior comprehension of diabetes pathogenesis, which thusly will prompt the improvement of new treatments for the illness. The use of viruses for inducing diabetes has become a new technique that can be used in testing anti-diabetic drugs. There are approximately 40.9 million diabetic subjects in India, according to the International Diabetes Federation (IDF).

KEYWORDS: Diabetes mellitus, *In vivo*, *In vitro*, cell line.

INTRODUCTION

Diabetes mellitus develops when the pancreas fails to generate an adequate quantity of insulin or when the body can not utilize insulin properly. It is a chronic metabolic disease that can elevate glucose levels. Prolonged hyperglycemia has detrimental effects on the organs and tissues of the body. Projections indicate that 552 million people worldwide will have diabetes by 2030, up from 366 million in 2011. Similarly, the Worldwide Diabetes Collaboration estimates that there are 183 million people who are completely unaware of this ailment.[1] The projections indicate that by 2030, the highest concentration of diabetes patients will be in India, China, and the United States.[2] Insulin-dependent diabetes mellitus, often known as Type-1 diabetes, is a condition when there is a complete lack of insulin. The beta cells undergo destruction as a result of viral invasion, exposure to harmful substances, or the presence of antibodies.[3] When an individual has Type 2 diabetes or non-insulin dependent diabetes mellitus, there is a significant likelihood of experiencing both endogenous and exogenous insulin resistance.[4] Type-3 diabetes, also known as maturation onset diabetes young (MODY), is a condition that arises from chronic pancreatitis or extended usage of medications such as glucocorticoids, thiazide diuretics, diazoxide, growth hormone, and some protease inhibitors. Gestational diabetes mellitus, also known as Type 4 diabetes, occurs during pregnancy. Placental hormones, which enhance insulin resistance, cause this condition. This condition is responsible for around 4–5% of all pregnancy cases.[5] The symptoms of diabetes mellitus encompass polyuria, polydipsia, polyphagia, weight loss, impaired eyesight, and numbness or tingling in the hands or feet.[6]

The advanced knowledge and thorough understanding of the causes and mechanisms of diabetes, as well as the discovery of new therapies, depend on research on diabetes using both *in vivo* and modern *in vitro* methods.[7] There are various experimental diabetic treatment options, such as pharmacological, surgical, and genetically engineered animals. An appropriate animal model and variable environmental conditions are required.[8] To assess new synthetic elements and other remedial methodologies for the treatment of diabetes, a suitable animal model is used.[9]

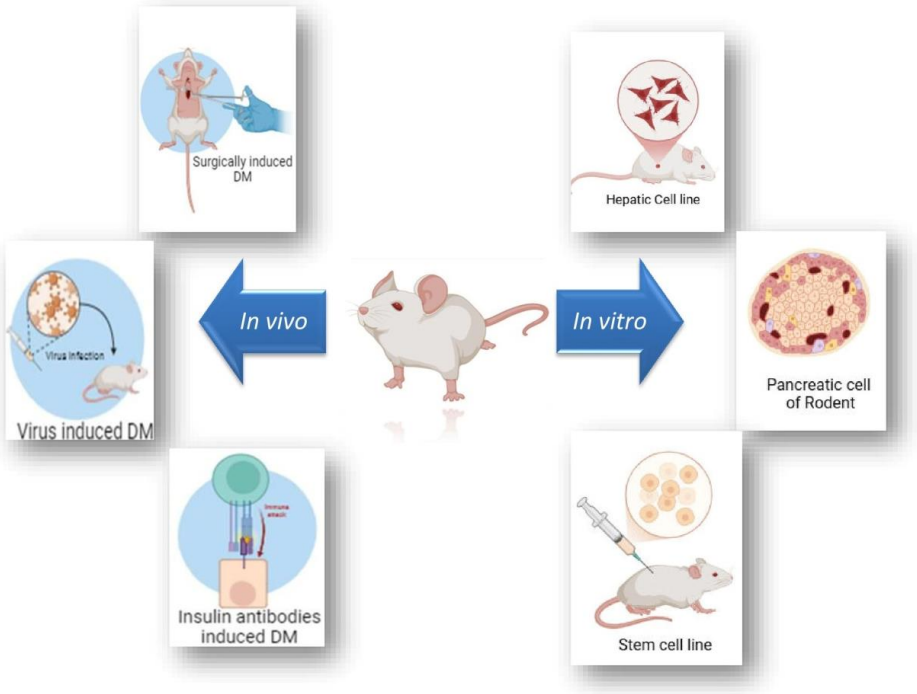

Figure 1: Graphical representation of *in vivo*, *in vitro*, and cell line techniques used in Diabetes model.

The main objective of the current work is to use *in vivo*, *in vitro*, and cell line techniques for diabetes research. Cell lines have shown many benefits in the field of research on anti-diabetic therapies. Foundational research must adhere to the 3Rs concept (replace, refine, and reduce). Cell lines are used to evaluate the anti-diabetic potential of various synthetic and natural medicines, replacing animal investigations. Cell culture is reliable because it allows for direct access and evaluation of the tissue.[10] The current review combines all *in vivo*, *in vitro* models, and cell lines into a single resource to assist researchers seeking experimental studies on diabetes.

The scientific articles were obtained from various bibliographic databases, such as Sci-Finder, PubMed, and Science Direct.

IN VIVO STUDIES IN ANTI DIABETIC ACTIVITY

Alloxan: Chemical Cause of Diabetes

Alloxan is the most notable substance utilized in examinations that cause diabetes. In research, Type 1 diabetes is induced. The urea metabolite Alloxan selectively necrosis the cells of pancreatic islets.[8] By altering the quantity of Alloxan utilized, researchers have produced effectively experimental diabetes in a variety of animal species, including rabbits, rats, mice, and dogs, with a variety of levels of disease activity.[11]

Streptozotocin: Chemical cause of Diabetes

In animal models, dosages of the naturally occurring chemical streptozotocin (STZ) cause Type 1 and Type 2 diabetes. Furthermore, in addition to its application in the treatment of metastatic islets of Langerhans cancer, it possesses several additional medical uses.[12]

Diathizone: Chemical Cause of Diabetes

Mice, rats, rabbits, and golden hamsters all have suffered from diabetes following the administration of Dithizone. Serum zinc, iron, and potassium levels are increased in diathizonized diabetic mice, but copper and magnesium levels fall. After receiving insulin therapy, the blood levels of potassium and magnesium return to normal.[13]

Gold thioglucose: Chemical Cause of Diabetes

Diabeticogenic compound, Gold thioglucose has been shown to actuate hyperphagia and serious heftiness prompting Type-2 diabetes in animals.[14]

Induced by monosodium glutamate, diabetes

The concentration of glutamate in the plasma is increased by monosodium glutamate (MSG). When it is activated the insulin is secreted. When mice is injected with MSG it develops hyperinsulinemia and obesity. This results in elevated levels of triglycerides, cholesterol, and blood sugar.[15,16]

Insulin antibodies induced diabetes

The antibodies of the insulin not only possess the ability to connect with it but also the affinity to do so. Postprandial hyperglycemia is a sign of postprandial insulin insufficiency because tissues do not get enough antibody bound insulin.[17,18]

The development of diabetes by ferric nitrilotriacetate

Animals that get a estimation of ferric nitrilotriacetate parenterally for 60 days giving symptoms of diabetes, similar to glycosuria, hyperglycemia, ketonemia, and ketonuria.[19] The chemical cause of diabetes and chemical properties are mentioned in Table - 1.

Table-1: Chemical cause of diabetes and its properties.

Sl. No.	Chemical cause of diabetes	Chemical properties
1	Alloxan	Oxygenated pyrimidine
2	Streptozotocin	Monofunctional nitrosourea
3	Diathizone	Organo sulfur
4	Gold thioglucose	Sugar glucose
5	Monosodium glutamate	Non-essential amino acid
6	Ferric nitrilotriacetate	Nitrilotriacetic acid

Virus Induced Diabetes

JDM might be due to infectious diseases and beta cell-specific autoimmunity.[20] In the 1960s, Bet and partners noticed an ascent in adolescent beginning diabetes (Type 1) because of viral contaminations. Right now, the Coxsackie infection and the D-kind of encephalomyocarditis (EMC-D) have been recognized as infections.[21]

D-Variation Encephalomyocarditis

By tainting and killing pancreatic beta cells in a few innate types of mice, the EMC-D infection can bring about insulin-subordinate hyperglycemia.[22] Pre-treatment with the powerful immunosuppressive medication cyclosporine- which spreads the degree and speed of diabetes in ICR Swiss mice.[23] Utsugi *et al.* presented the NDK25 EMC-D viral clone in 1992. Intraperitoneal injection of NDK25 causes the development of non-insulin-dependent diabetes mellitus.[24]

Coxsackie Viruses

Coxsackie infections can cause diabetes in mice and infect and kill pancreatic acinar cells while sparing the nearby islets of Langerhans. There is a strong correlation between coxsackie B4 infection and the amelioration of insulin-dependent diabetic mellitus in individuals. The disease that outcomes in diabetes is brought by Coxsackie infection which contaminates and aggravates tissue harm, and the production of sequestered islet antigen that invigorates resting auto-receptive Lymphocytes, further directing the islet antigen refinement in an aberrant result of the viral diseases.[25,26]

Hormone induced Diabetes

Growth hormone induced diabetes

Higher experimental animals get growth hormone repeatedly to induce diabetes with ketonuria and ketonemia. Beta cells and islet tissues were destroyed as a result of long-term growth hormone usage, which also caused by persistent diabetes.[27]

Corticosteroid initiated diabetes

Steroid diabetes is the post- effect of corticosteroid. Steroid diabetes is brought on by dexamethasone and prednisolone. The liver's gluconeogenesis is accelerated by glucocorticoids, boosting hepatic glucose and causing insulin resistance and hyperglycemia.[28]

Surgically Induced Diabetes

It involves the removal of the pancreas in order to cause diabetes.[29] Mild to moderate hyperglycemia is treated with pancreatectomy.[30]

Genetically modified diabetic mice

Significant advancements have been made in the sector recently as a consequence of the introduction of transgenic mice. Glucose metabolism may be influenced by rodents that have either over (transgenic) or under (knockout).[31,32] Natural products and the models have not yet subjected to protocols. Their research may be restricted by the high expenses of convoluted techniques that examine the mechanisms of possible therapeutic drugs.[33]

Independently Obese Diabetic Rodent Models

Mouse ob/ob

The leptin quality is upset in the ob/ob mouse breed and the leptin consumption causes serious insulin opposition.[34,35] The strain ob/ob inhibits increase in body weight, insulin resistance, and hyperinsulinemia.[36] Initial 3- 4 weeks of life span they are set apart by hyperinsulinemia, insulin opposition, and hyperphagia in the ob/ob model. As mice age, they show signs of Type 2 diabetes with regular declines in plasma insulin levels.[37,38]

Mouse db/db

When C57BL/KsJ mice are deficient in leptin receptors, db gene mutations occur spontaneously. Mutations on chromosome number 4 led to the strain's emergence.[39] Within 2 weeks of birth, db/db mice develop hyperphagia, hyperinsulinemia, and insulin resistance and between the ages of 3-4 weeks, obesity first manifests, followed by hyperglycemia between the ages of 4- 8 weeks.[24] Microvascular complications of diabetes and renal failure are studied using the db/db mouse.[40,41]

Mouse Kuo Kondo

The obese and Type 2 Diabetes model is the Kuo Kondo (KK) mouse.[42] The KK mouse exhibits obesity, hyperinsulinemia, and hyperglycemia instinctively.[43] Insulin resistance, compensatory hyperinsulinemia, and hyperphagia is manifested to the KK mouse's development of obesity at the age of 2 months old. According to the study, the peak of insulin resistance and hyperinsulinemia, happened around 5 months.[44]

Rat Zucker Diabetic Fatty (ZDF)

Zucker Diabetic Fatty (ZDF) rats have reduced strength, increased insulin sensitivity, and a heightened predisposition to diabetes due to inadequate insulin production.[45] At 12 weeks, the male ZDF rat develops into diabetic symptoms. Ordinary peak serum insulin levels occur at around 7 to 10 weeks, the stimulus of glucose and the level of insulin cannot respond to it.[46]

New Zealand Obese (NZO) mouse

At 10 weeks old enough, weight gain is promoted by hyperphagia, hyperglycemia, and hyperinsulinemia in the New Zealand type of fat mice.[47] Insulin obstruction initially shows up in the NZO mouse on very early age by the time the NZO mouse is 20-24 weeks old, blood glucose levels are increased to 300-400 mg/dL as hyperglycemia and glucose tolerance increases with age.[48] Studies of obesity and diabetes can be conducted using this model.[49]

Otsuka Long-Evans, Tokushima, Fatty (OLETF) rat

The OLETF rat shows hyperglycemia between 18 and 25 weeks of age. Obesity, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, and hypertriglyceridemia are all present in OLETF rats, just as they are in people with Type 2 DM. There are various passive qualities associated with actuating diabetes in OLETF rodents, remembering a few qualities for the X chromosome.[50,51]

Mouse from Nagoya-Shibata-Yasuda (NSY)

The NSY mouse model closely resembles Type 2 diabetes in humans by having characteristics like a healthy weight, less insulin production, and insulin resistance, all of which help diabetes get worse over time. Just around 30% of female NSY mice foster diabetes, contrasted with 100% of males.[52]

Obese Diabetes Mouse Model by Tsumura

At 2 years old, the TSOD (Tsumura, Suzuki, Obese Diabetes) mouse exhibits weight gain and insulin resistance, leading to hyperinsulinemia and hyperglycemia. There is a pancreatic islet hypertrophy in TSOD mice. The broken GLUT4 movement in adipocytes and skeletal muscle of the TSOD mice brings about decreased insulin responsiveness and insulin opposition.[53]

Mouse M16

M16 mice are obese throughout their lives. All M16 mice have hyperglycemia, hyperinsulinemia, and hypercholesterolemia at 8 weeks of age.[55]

Spontaneous Diabetic Non-Obese Rodent Models

Cohen diabetic rat

By putting the rat on a manufactured 72% sucrose copper-poor diet routine for 2 months, a hereditary model of Type 2 DM was made. The symptoms present Insulin resistance, hyperinsulinemia, and non-obesity. The Cohen diabetic rat exhibits a hereditary propensity for a diet high in carbohydrates, which is a sign of Type 2 DM in people.[56]

The GK rat (Goto Kakizaki)

The GK rodent is a non-stout T2DM model with insulin obstruction, hyperinsulinemia, and hyperglycemia. GK rodents had a diligent fasting hyperglycemia towards the finish of the initial 14 days. Following 2 months, hyperglycemia turns out to be less serious, and glucose invigorates the islets to deliver insulin. In GK rats, diabetic complications include retinopathy and peripheral neuropathy.[57,58,59]

Spontaneous Diabetes Torii rat

A recently immediately non-stout diabetic strain is the SDT (Spontaneously Diabetic Torii) rodent.[60] It exhibits traits such as hyperglycemia, hyperinsulinemia, glucose intolerance, and hypertriglyceridemia.[61] SDT rats have significant hyperglycemia, which causes them to develop diabetic retinopathy,[62] diabetic nephropathy and neuropathy. This model is appropriate for researching human T2DM problems.[63,64]

IN VITRO STUDIES IN ANTI DIABETIC ACTIVITY

Assay of α Amylase Inhibition *in Vitro*

The measurement of amylase activity was conducted by utilizing 1% concentration of soluble starch as the substrate in a sodium phosphate buffer with a pH of 6.9 and M of 0.02 mol/L. The extracts with concentrations of 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL were mixed with a substrate solution that was made to a total volume of 150 µL. A volume of 10 µL of amylase solution with a concentration of 1 unit/ mL /min. was introduced. Following incubation at a temperature of 250 °C for a duration of 30 min., introduce 30 µL of dinitro salicylic acid reagent and incubate at a temperature of 90 °C for a duration of 5 min. The measurement of absorbance was conducted at a wavelength of 540nm.[65]

Assay of α Glucosidase Inhibition *in Vitro*

Along with 150 µL of 0.1 mol/L potassium phosphate buffer (pH 6.9), the extracts were mixed in 4 different amounts: 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL. Additionally, 10 µL of glucosidase solution (1 unit/mL/min) was added. The mixer was placed in an incubator and maintained at a temperature of 37 °C for a duration of 15 min. This was followed by adding 10 mL of a solution that contained 3 mM/L of p-nitrophenyl D-glucopyranoside (PNP-G). The mixture was then left to sit for another 10 min. at 37 °C. The reaction was terminated by introducing 30 mL of a 0.1 M solution of sodium carbonate. A UV spectrophotometer measures the amount of product (p-nitrophenol) produced at a wavelength of 405 nm to assess the enzyme's activity.[66]

In-vitro studies on insulin secretion and glucose uptake

Oral anti-diabetic medications have the potential to impact numerous glucose metabolism pathways, including insulin production, target organ glucose uptake, and food absorption. Recent therapies focus on transcription factors such peroxisome proliferator-activated receptors (PPAR) and incretin. People have not concentrated on treating diabetes without using insulin receptors or glucose transporters.[67,68]

A VARIETY OF CELL LINES ARE AVAILABLE FOR ANTIDIAETIC ACTIVITY

A cell line is a collection of cells or tissues derived from a single parent cell. *In vitro* cell culture involves culturing cell lines in tubes, flasks, or plates with an appropriate growth media. When comparing cell lines with newly produced cells, known as "primary" cells, it is important to note that gene expression in primary cells may differ from individual cells, whereas cell lines consisting of several cells that are genetically similar.

1. Isolated islet cell lines from the pancreas

Various *in-vitro* tests are accessible to investigate the different phases of insulin creation, as it was recently observed. Using glucose, pancreatic cells convert ADP into ATP. The primary function of glucose is the direct stimulation of acid secretion. When the ATP/ADP ratio goes up in the cytoplasm, the potassium channels close. This makes the cell membrane less charged and turns on voltage-dependent Ca^{2+} channels. This initiation causes the release of insulin, which raises the Ca^{2+} level inside the cell. Using isolated pancreatic cells digested with collagenase either from diabetic or control rats or mice, the pattern of insulin release is examined. The proper transfer and separation method of the designated culture medium are then performed. Later, this experimental methodology is evaluated.[69]

Insulin-secreting cell lines

Researchers using natural compounds are primarily interested in inhibiting insulin secretion and cell dysfunction. These explorations have become more successful because of the use of bioengineered advancements, which have further developed refined cell lines for the help of discoveries under their technique and methods. HIT, MIN6, INS-1, RIN and beta-TC cells are examples of commonly used insulin-emitting cell lines. Due to their propensity to release trace quantities of somatostatin, glucagon and insulin, these cell lines are often used in research. They don't exactly mirror primary cell physiology's activity. They continue to demonstrate that they are crucial to the investigation of cell dysfunction.[70]

Insulinoma cell line from rats

RINr and RINm were the primary cell lines obtained from rat development. Rodent insulinoma created by high portion X-beam illumination was utilized to foster RINm5F cells; these cells vary from corresponding cells with regards to their characteristics. The most popular insulin-secreting cell line is called RIN. In addition to some quantity of glucagon and somatostatin, they mostly contain insulin. Moreover, RINr and RINm5F exhibit the traits of phosphorylation and glucose sensitivity.

Insulinoma cell line (INS-1)

Significant traits of INS-1, such as having a high insulin content, are analogous to those of the pancreatic cell. Rat insulinoma were exposed to X-rays to create INS-1 cells. These cells produce proinsulin I and proinsulin II. The primary drawback of these cells is that their culture medium must contain mercaptoethanol. The protein can be denatured by the hazardous chemical mercaptoethanol. These cells cannot be multiplied without the use of mercaptoethanol, and they risk losing a number of crucial properties.

Pancreatic beta cells from hamsters

HIT-T15 cells were produced using beta cells from hamster pancreas. Simian virus 40 transformation is the manufacturing method. They are made up of membrane-bound secretory granules that resemble hamster cell features. Immunizer immunofluorescence staining is utilized to recognize the presence of insulin. Conversely, with hamster cells, the insulin content/mg protein is 2.5-20 times on various occasions lower in these cells.[10] It is proposed as a clonal cell line. The clonal cell line was initially identified in a hamster pancreatic islet, where its primary relevance is in its reaction to glucose, since it may make insulin, which reduces insulin levels. The HIT-T15 cell line utilizes an assortment of exploration media, including 11.1 M glucose. Cells are continuously grown in high-glucose conditions while insulin levels go up. A gene mutation reduces the amount of mRNA and causes a shortfall of the pancreatic islet glucose transporter. The glucose concentration in this cell line is lowered. The HIT-T15 cell line has a greater physiological impact.[71]

2. Adipocyte cell line

Since its disclosure, the notable cell line 3T3 has been utilized as a model framework in a few examinations. Because of its changed attributes, including glucose homeostasis and lipid stockpiling, 3T3-L1 is suggested over 3T3-F442A for against diabetic examinations. Pre-adipocytes differentiate into 3T3-L1 adipocytes throughout this process. The adipocytes found a 20-fold increase in insulin receptors throughout this transition. As a result, they produce the capacity to use glucose in response to insulin. By functioning as a regulator and accelerating the process, insulin is also essential in this differentiation process.[72]

3T3 -L1 adipocytes

About 30 years ago, researchers Kehinde and Green discovered that mouse 3T3 cells cloned in the "resting state" showed a propensity to develop into adipocytes. Adipogenesis is studied using a 3T3-L1 cell culture paradigm to examine a number of systems, such as adipocyte differentiation, lipid metabolism, glucose transport, and insulin signaling. This technique may comparably be utilized to find enormous adipocyte-delivered proteins including adiponectin, resistin, and visfatin. The 3T3 cell line was disconnected from the animal after it went through update affected improvement from fibroblasts to make adipocytes through halfway arrangement of pre-adipocytes that had high extents of TAG.

The 3T3-L1 cell line is cultivated at entry in 96-well plates at a thickness of 5×10^3 cells/well. After that, oil is replaced stained is performed. The medium consisting of 10% FBC in DMEM/F12 is used to assess glucose absorption. After 2 days of confluence, differentiation media are added to the present mixture. These media contain DMEM/F12 + 2% FBS, 10 g/mL insulin, 0.5 mM (IBMX) 3-isobutyl-1-methylxanthine, and 1.0 M dexamethasone. In something like 5 days of treatment with isobutyl methyl xanthine, insulin, and dexamethasone, the 3T3cell lines show a round aggregate and start to gather intracellular lipids. After replacing the detachment medium with media for 4 days, introduce DMEM/F12 supplemented with 2% FBS.[73]

Mesenchymal stem cell line

Adipocyte, myoblast, and chondrocyte separation is a trait of mesenchymal undifferentiated cells. It can separate into adipocytes with the guide of PPAR (Peroxisome proliferator-activated receptors) gamma receptors. NAD (Nicotinamide Adenine Dinucleotide) is a protein that is reliant on PPAR gamma.[74] Many substances, including the plant polyphenol resveratrol, are regulated by PPAR gamma. A mouse cell line makes up this cell line. The primary growth media for the cell line contains L-ascorbic acid, α-glycerophosphate, dexamethasone, and other additives. This cell line's main job is to monitor anti-diabetic action. Adipocyte production mostly uses this kind of cell line. Quantitative continuous PCR and ANOVA measurable examination are the two primary methods for quickly evaluating the declaration of markers in mesenchymal immature microorganism lines. MSC (Mesenchymal stem cell) cell lines, which show different sorts of mesenchymal linkage, are tracked down in essential bone marrow. This kind of cell line makes up a sizable piece of macrophages and significantly lesser piece of myeloid and endothelial cells. The mesenchymal cells are multipotent primary microorganisms.[75]

3. Muscle cell line (Skeletal muscle)

Cellular line L6

The L6 cell line is a rodent myoblast cell line. The cell culture medium should contain penicillin, streptomycin, amphotericin B, 10% bull like serum, and 5% FBS. The temperature is maintained at 37° C, while the dampness is kept at 5% CO_2.[76] TPVG (Trypsin Phosphate Versene Glucose) arrangement, 0.05 glucose, and 0.02% EDTA separate from this cell line. Prior to being kept on a 96 well plate, the way of life should develop into a 25 cm culture flax. The most important model is L6, which regulates *in vitro* muscle myogenesis. In addition to determining signal transduction, the L6 cell model also predicts cell destiny and promotes cell growth. In healthy condition, the mononucleate L6 cell line develops into a multinucleate myotube. Myotubes in this cell line exhibit certain morphological traits.[77]

Cellular line BMS2

The bone marrow stromal cell line (BMS2) was identified in mouse bone marrow. The primary media used for culturing the BMS2 cell line are DMEM and supplements enriched with 10% FBS. A paradigm for multi-potent stromal stem cells is provided by this cell line.[69] Models of myelopoiesis and lymphopoiesis also support BMS2 cell line. A microenvironment is created by vitamin D in the layers of stromal cells that are densely packed with adipocytes in BMS2. It usually takes three days to produce bone marrow cell culture media, which also acts as an adipogenic agent.[78]

BC3H– 1 cell line

This cell line was made by a researcher Shubert in 1974. The cell line primarily regulates muscle-related protein and helps in muscle separation. Bc3H-1 essentially works as a development factor for separation from the way of life medium. This cell line was first found in neoplastic mouse cerebrum tissue. This cell line's morphological and physiological qualities consolidate smooth muscle starting. In this cell line, nicotinic Ach and MCK receptors are accessible. The separation and guideline of the muscle-explicit quality are the primary objectives of the Bc3H-1 cell line. This cell line's ability is to disclose the genesis of skeletal muscles and the expression of phenotypic genes particular to each kind of muscle which is vital to bear in mind.[79] Serum-derived media is added during the Bc3H-1 cell line's incubation period. Nicotinic Ach and MCK receptors are tracked down in this cell line. The significant goals of the Bc3H-1 cell line are separation and control of the muscle-explicit quality. It is critical to recollect that this cell line has the ability to uncover the beginning of skeletal muscles and the statement of phenotypic qualities exceptional to every sort of muscle.[80]

4. Hepatic cell lines

This cell line makes a sensible *in-vitro* model system since it was made in fetal cow-like serum. The cell line requires a steady 37°C temperature, a CO_2 supply, and a stickiness level of 100%. The culture media needs to be replaced twice a week for the cell line, which is cultivated on a weekly basis. EDTA is used to separate hepatic cell lines, and a single-cell solution is created. The quantity of suitable cells is resolved utilizing a hemocytometer.[81] To get the ideal cell thickness of 105 cells/mL, the medium is consequently weakened with 5% FBS. A 96-well plate is utilized to hold the pre-arranged cell suspension before it is cultured. The temperature and air tenacity are stayed aware of while cell lines are treated for 24 hr. The cell lines are then moreover debilitated in (stimulating organs) serum media resulting to being broken down in dimethyl sulfoxide (DMSO). Various obsessions are plated to achieve a last combination of 31.25, 62.5, 125, 250, and 500 ug/mL All focuses are kept up with in three-fold. Hepatic cell line is investigated using phase contrast and ultraviolet-emitted fluorescence microscopy after 48 hr. of incubation.[82]

Cellular line HEP G2

A human liver malignant growth cell line is otherwise called Hep G2 cell line. From liver tissue, the majority of Hep G2 cell lines are inferred. The boy, an American teenager who is Caucasian, possesses this cell line. The 96-well plate was used for the tests, while the culture flask was used to cultivate the stock cultures.[83] Hepatocellular carcinomas is the primary differentiation of these cell lines. The cell line has an epithelial morphology. Among this cell line's chromosomes, there are 55.[84] The *in-vitro* model system is appropriate for this cell line analysis. At the point when seen under a magnifying instrument, the Hep G2 cell lines have all the earmarks of being strong. This cell line is additionally present in the basolateral and apical cell surface areas of practical separation. Hep G2 is for the most part

utilized in drug examination and disease cell tests. Promptly it is basically performed in the morning time and the point at which the cell line is accumulated. The medium is joined with DMSO, bringing the last fixation down to under 0.5 V/V. The device is used to maintain the cell line.[85]

Research utilizing distinct pancreatic islet cell lines

It is generally alluded to that pancreatic cells produce insulin due to adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The extension in the cytoplasmic ATP/ADP extent makes the plasma layer depolarize, blocking ATP-fragile potassium channels and activating voltage-dependent Ca^{2+} channels. Thus, the intracellular Ca^{2+} fixation rises, setting off insulin emission starts.[86]

Glucose production test with H4IIE

A streptomycin or penicillin solution is added if contamination is found in the test. Lactate and pyruvate measurements for the generation of glucose are used to calculate the cell count. 100 mm plate has been predominately filled with cells. The cells are exposed to a solution containing 500 mm of dexamethasone, 10 mm of insulin, herbal aqueous, and 0.1 m of Mc AMP. Once again, 1 mL of a glucose synthesis catalyst mixture and 0.3 mL of a glucose analysis solution are introduced to the prepared solution and incubated for a further 3 hr. to detect glucose. The generation of glucose impacts the expression of the glucose production % in the medium control, which in turn affects the total quantity of cell proteins.[87]

CONCLUSION

Diabetes mellitus, is the most well-known and disorder metabolic ailment, is perceived as one of the worldwide general medical conditions with the quickest speed of rise. This paper discusses several in vitro methodologies and animal models that mimic the qualities and characteristics of human diabetes. Diabetes research has utilized a range of experimental animal models. Each is unique in terms of both the disorder's characteristics and course of development. The pharmacology and processes behind the metabolic disease are frequently studied using experimental models. Diabetic models demonstrate the recurrence and severity of this infection, which will allow insight into diabetes' etiology and repercussions. Since diabetes is the leading cause of retinopathy, nephropathy, cardiomyopathy, and neuropathy worldwide. In spite of the multitude of advantages these exploratory models might accommodate exploring and making new, sensible drugs, every one of them has novel restricts that will likewise influence how new drugs and remedial medicines are planned. Therefore, it is crucial to choose a model that has all the qualities required to be an ideal model, which vary based on the problems of diabetes and the chosen animal. In order to accurately portray the many difficulties experienced by human diabetic patients, experimental animal models are used, which necessitates careful consideration and research when choosing the best animals for the study.

ACKNOWLEDGEMENT

The authors are thankful to the School of Pharmaceutical Sciences, Siksha 'O' Anusandhan, deemed to be University Bhubaneswar, Odisha for providing continuous support and encouragement in the promotion of scientific works.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DM: Diabetes mellitus; IDF: International Diabetes Federation; 3Rs: Replace, Refine, Reduce; STZ: Streptozotocin; MSG: Monosodium glutamate; JDM: Juvenile diabetes mellitus; EMC-D:

D-kind of encephalomyocarditis; ICR: Insulin to carbohydrate ratio; KK: Kuo Kondo; ZDF: Zucker diabetic fatty; NZO: Obese New Zealand; OLETF: Long-Evans Otsuka, Tokushima, fat; NSY: Nagoya-Shibata-Yasuda; TSO: Tsumura, Suzuki, Obese Diabetes; GLUT4: Glucose transporter type 4; GK: Goto Kakizaki; T2DM: Type 2 diabetes mellitus; SDT: Spontaneously Diabetic Torii

PNP-G: P-nitrophenyl D-glucopyranoside; UV: Ultra violet; PPAR: peroxisome proliferator-activated receptors; ADP: Adenosine diphosphate; ATP: Adenosine tri-phosphate; INS-1: Insulinoma cell line; FBC: Full blood count; DMEM/F12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; IBMX: Isobutyl-1-methylxanthine; TPVG: Trypsin Phosphate Versene Glucose; EDTA: Ethylene diamine tetra acetic acid; NAD: Nicotinamide Adenine Dinucleotide; PCR: Polymerase chain reaction; ANOVA: Analysis of variance; MSC: Mesenchymal stem cell; FBS: Fasting blood sugar; DMSO: Dimethyl sulfoxide;BMS2:Bone marrow stromally

REFERENCES

1. Federation ID. One adult in ten will have diabetes by 2030.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care. 2004 May 1; 27(5): 1047-53.
3. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'donnell CJ. Metabolite profiles and the risk of developing diabetes. Nature medicine. 2011 Apr; 17(4): 448-53.
4. Mishra G, Panda PK, Satapathy T. Animal models for type 2 diabetes: A review. World Journal of Pharmaceutical Sciences. 2016 Jan 1: 141-7.
5. Tripathi V, Verma J. Current updates of Indian antidiabetic medicinal plants. Int J Res Pharm Chem. 2014; 4(1): 114-8.
6. Riaz S. Review of Diabetes mellitus. Academic Journals. 2009; 4(5): 367-73.
7. Dewangan H, Tiwari RK, Sharma V, Shukla SS, Satapathy T, Pandey R. Past and Future of in-vitro and in-vivo Animal Models for Diabetes: A Review. Indian Journal of Pharmaceutical Education and Research. 2017 Oct 1; 51(4S): s522-30.
8. Etuk EU. Animals models for studying diabetes mellitus. Agric Biol JN Am. 2010 May 12; 1(2): 130-4.
9. Sen K, Satapathy T. Efficient and Safe Induction of Diabetes in Experimental Animals: A Review on Alternative Models and Techniques. Journal of Lab Animal Research. 2024; 3(5): 27-39.
10. Goswami RB, Goswami N, Khare P, Choudhary S, Pathak AK. Evaluation of Anti-Diabetic Activity of Leaves and Fruits of Ficus religiosa Linn. Research Journal of Pharmacognosy and Phytochemistry. 2010; 2(1): 61-63.
11. Iranloye BO, Arikawe AP, Rotimi G, Sogbade AO. Anti-diabetic and anti-oxidant effects of Zingiber officinale on alloxan-induced and insulin-resistant diabetic male rats. Nigerian journal of physiological sciences. 2011; 26(1): 89-96.
12. Brentjens R, Saltz L. Islet cell tumors of the pancreas: the medical oncologist's perspective. Surgical Clinics of North America. 2001 Jun 1; 81(3): 527-42.

13. Halim D, Khalifa K, Awadallah R, El-Hawary Z, El-Dessouky EA. Serum mineral changes in dithizone-induced diabetes before and after insulin treatment. Zeitschrift für Ernährungswissenschaft. 1977 Mar; 16: 22-6.
14. Karasawa H, Takaishi K, Kumagae Y. Obesity-induced diabetes in mouse strains treated with gold thioglucose: a novel animal model for studying β-cell dysfunction. Obesity. 2011 Mar; 19(3): 514-21.
15. Kim MR, Sheeler LR, Mansharamani N, Haug MT, Faiman C, Gupta MK. Insulin antibodies and hypoglycemia in diabetic patients: can a quantitative analysis of antibody binding predict the risk of hypoglycemia?. Endocrine. 1997 Jun; 6: 285-91.
16. Chung JO, Cho DH, Chung DJ, Chung MY. Spontaneous hypoglycemia due to insulin antibody after insulin treatment of diabetic ketoacidosis. Endocrinology and Metabolism. 2010 Sep 1; 25(3): 217-20.
17. Nagata M, Suzuki W, Iizuka S, Tabuchi M, Maruyama H, Takeda S, Aburada M, Miyamoto KI. Type 2 diabetes mellitus in obese mouse model induced by monosodium glutamate. Experimental Animals. 2006; 55(2): 109-15.
18. Van Haeften TW, Bolli GB, Dimitriadis GD, Gottesman IS, Horwitz DL, Gerich JE. Effect of insulin antibodies and their kinetic characteristics on plasma free insulin dynamics in patients with diabetes mellitus. Metabolism. 1986 Jul 1; 35(7): 649-56.
19. Le Marchand-Brustel Y, Jeanrenaud BE, Freychet PI. Insulin binding and effects in isolated soleus muscle of lean and obese mice. American Journal of Physiology-Endocrinology and Metabolism. 1978 Apr 1; 234(4): E348.
20. Le Marchand-Brustel Y. Molecular mechanisms of insulin action in normal and insulin-resistant states. Experimental and clinical endocrinology & diabetes. 1999; 107(02): 126-32.
21. Craighead JE. Current views on the etiology of insulin-dependent diabetes mellitus. New England Journal of Medicine. 1978 Dec 28; 299(26): 1439-45.
22. Gamble DR, Kinsley ML, FitzGerald MG, Bolton R, Taylor KW. Viral antibodies in diabetes mellitus. Br Med J. 1969 Sep 13; 3(5671): 627-30.
23. Yoon JW, McClintock PR, Onodera T, Notkins AL. Virus-induced diabetes mellitus. XVIII. Inhibition by a nondiabetogenic variant of encephalomyocarditis virus. The Journal of experimental medicine 1980; 152(4): 878-92
24. Gould CL, McMannama KG, Bigley NJ, Giron DJ. Virus-induced murine diabetes: enhancement by immunosuppression. Diabetes. 1985 Dec 1; 34(12): 1217-21.
25. Utsugi T, Kanda T, Tajima Y, Tomono S, Suzuki T, Murata K, Dan K, Seto Y, Kawazu S. A new animal model of non-insulin-dependent diabetes mellitus induced by the NDK25 variant of encephalomyocarditis virus. Diabetes research (Edinburgh, Scotland). 1992 Jan 1; 20(4): 109-19.
26. Lansdown AB, Brown JD. Immunization of mice against Coxsackievirus B3 and prevention of foetal growth retardation. British journal of experimental pathology. 1976 Oct; 57(5): 521.
27. Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvennick N. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. Nature medicine. 1998 Jul 1; 4(7): 781-5.
28. Moloney PJ, Coval M. Antigenicity of insulin: diabetes induced by specific antibodies. Biochemical journal. 1955 Feb; 59(2): 179.
29. Yoon JW. The role of viruses and environmental factors in the induction of diabetes. Current topics in microbiology and immunology. 1990 Jan 1; 164: 95-123.
30. Sangeetha R, Vedasree N. In Vitro α-Amylase Inhibitory Activity of the Leaves of Thespesia populnea. International Scholarly Research Notices. 2012; 2012(1): 515634.

31. Ramachandran S, Rajasekaran A, Adhirajan N. In vivo and in vitro antidiabetic activity of Terminalia paniculata bark: an evaluation of possible phytoconstituents and mechanisms for blood glucose control in diabetes. International Scholarly Research Notices. 2013; 2013(1): 484675.
32. Ueda H, Ikegami H, Yamato E, Fu J, Fukuda M, Shen G, Kawaguchi Y, Takekawa K, Fujioka Y, Fujisawa T, Nakagawa Y. The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. Diabetologia. 1995 May; 38: 503-8.
33. Hirayama I, Yi Z, Izumi S, Arai I, Suzuki W, Nagamachi Y, Kuwano H, Takeuchi T, Izumi T. Genetic analysis of obese diabetes in the TSOD mouse. Diabetes. 1999 May 1; 48(5): 1183-91.
34. Miao G, Ito T, Uchikoshi F, Tanemura M, Kawamoto K, Shimada K, Nozawa M, Matsuda H. Development of Islet-Like Cell Clusters After Pancreas Transplantation in the Spontaneously Diabetic Torri Rat. American journal of transplantation. 2005 May 1; 5(10): 2360-7.
35. Elmquist JK, Maratos-Flier E, Saper CB, Flier JS. Unraveling the central nervous system pathways underlying responses to leptin. Nature neuroscience. 1998 Oct; 1(6): 445-50.
36. Shafrir E. Animal models of non-insulin-dependent diabetes. Diabetes/metabolism reviews. 1992 Oct; 8(3): 179-208.
37. Coleman DL. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia. 1978 Mar; 14: 141-8.
38. Jaidane H, Hober D. Role of coxsackievirus B4 in the pathogenesis of type 1 diabetes. Diabetes & metabolism. 2008 Dec 1; 34(6): 537-48.
39. Notkins AL. Virus-induced diabetes mellitus. Archives of Virology. 1977 Mar; 54: 1-7.
40. CAMPBELL J, Chaikof L, Davidson IW. Metahypophyseal diabetes produced by growth hormone. Endocrinology. 1954 Jan 1; 54(1): 48-58.
41. Ferris HA, Kahn CR. New mechanisms of glucocorticoid-induced insulin resistance: make no bones about it. The Journal of clinical investigation. 2012 Nov 1; 122(11): 3854-7.
42. Elmquist JK, Maratos-Flier E, Saper CB, Flier JS. Unraveling the central nervous system pathways underlying responses to leptin. Nature neuroscience. 1998 Oct; 1(6): 445-50.
43. Shafrir E. Animal models of non-insulin-dependent diabetes. Diabetes/metabolism reviews. 1992 Oct; 8(3): 179-208.
44. Coleman DL. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia. 1978 Mar; 14: 141-8.
45. Bates SH, Kulkarni RN, Seifert M, Myers MG. Roles for leptin receptor/STAT3-dependent and-independent signals in the regulation of glucose homeostasis. Cell metabolism. 2005 Mar 1; 1(3): 169-78.
46. Lee SM. Experimental diabetic nephropathy in the dbldb mouse. Dietary and pharmacologic therapy. 1984; 1: 419-24.
47. Hg V. Drug discovery and evaluation. Pharmacological assays. 1997: 360-420.
48. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T. Spontaneous long-term hyperglycemic rat with diabetic complications: Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes. 1992 Nov 1; 41(11): 1422-8.
49. Kawano K, Hirashima T, Mori S, Natori T. OLETF (Otsuka Long-Evans Tokushima Fatty) rat: a new NIDDM rat strain. Diabetes research and clinical practice. 1994 Oct 1; 24: S317-20.

50. Ueda H, Ikegami H, Yamato E, Fu J, Fukuda M, Shen G, Kawaguchi Y, Takekawa K, Fujioka Y, Fujisawa T, Nakagawa Y. The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. Diabetologia. 1995 May; 38: 503-8.
51. Hirayama I, Yi Z, Izumi S, Arai I, Suzuki W, Nagamachi Y, Kuwano H, Takeuchi T, Izumi T. Genetic analysis of obese diabetes in the TSOD mouse. Diabetes. 1999 May 1; 48(5): 1183-91.
52. Suzuki W, Iizuka S, Tabuchi M, FUNO S, YANAGISAWA T, KIMURA M, SATO T, ENDO T, KAWAMURA H. A New Mouse Model of Spontaneous DiabetesDerived from ddY Strain. Experimental Animals. 1999; 48(3): 181-9.
53. Kennedy JW, Hirshman MF, Gervino EV, Ocel JV, Forse RA, Hoenig SJ, Aronson D, Goodyear LJ, Horton ES. Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. Diabetes. 1999 May 1; 48(5): 1192-7.
54. Goto Y, Kakizaki M, Masaki N. Production of spontaneous diabetic rats by repetition of selective breeding. The Tohoku journal of experimental medicine. 1976; 119(1): 85-90.
55. Miralles F, Portha B. Early development of beta-cells is impaired in the GK rat model of type 2 diabetes. Diabetes. 2001 Feb 1; 50(suppl_1): S84.
56. Kondo K. Inbred strains resulting from Japanese mice. Bull Exp Anim. 1957; 6: 107-12.
57. Sato N, Komatsu K, Kurumatani H. Late onset of diabetic nephropathy in spontaneously diabetic GK rats. American journal of nephrology. 2003 Oct 1; 23(5): 334-42.
58. Yagihashi S, TONOSAKI A, YAMADA KI, KAKIZAKI M, GOTO Y. Peripheral neuropathy in selectively-inbred spontaneously diabetic rats: electrophysiological, morphometrical and freeze-replica studies. The Tohoku Journal of Experimental Medicine. 1982; 138(1): 39-48.
59. Sone H, Kawakami Y, Okuda YA, Sekine Y, Honmura S, Matsuo K, Segawa T, Suzuki H, Yamashita K. Ocular vascular endothelial growth factor levels in diabetic rats are elevated before observable retinal proliferative changes. Diabetologia. 1997 May; 40: 726-30.
60. Shinohara M, Masuyama T, Shoda T, Takahashi T, Katsuda Y, Komeda K, Kuroki M, Kakehashi A, Kanazaw Y. A new spontaneously diabetic non-obese Torii rat strain with severe ocular complications. Journal of Diabetes Research. 2000; 1(2): 89-100.
61. Ohta T, Matsui K, Miyajima K, Sasase T, Masuyama T, Shoda T, Koizumi H, Shinohara M, Matsushita M. Effect of insulin therapy on renal changes in spontaneously diabetic Torii rats. Experimental Animals. 2007; 56(5): 355-62.
62. Masuyama T, Fuse M, Yokoi N, Shinohara M, Tsujii H, Kanazawa M, Kanazawa Y, Komeda K, Taniguchi K. Genetic analysis for diabetes in a new rat model of nonobese type 2 diabetes, Spontaneously Diabetic Torii rat. Biochemical and biophysical research communications. 2003 Apr 25; 304(1): 196-206.
63. Choi SooBong CS, Park ChunHee PC, Choi MiKyung CM, Jun DongWha JD, Park SunMin PS. Improvement of insulin resistance and insulin secretion by water extracts of Cordyceps militaris, Phellinus linteus, and Paecilomyces tenuipes in 90% pancreatectomized rats.
64. Masiello P. Animal models of type 2 diabetes with reduced pancreatic β-cell mass. The international journal of biochemistry & cell biology. 2006 May 1; 38(5-6): 873-93.
65. El Adaouia Taleb R, Djebli N, Chenini H, Sahin H, Kolayli S. In vivo and in vitro anti-diabetic activity of ethanolic propolis extract. Journal of food biochemistry. 2020 Jul; 44(7): e13267.

66. Wu C, Li Y, Chen Y, Lao X, Sheng L, Dai R, Meng W, Deng Y. Hypoglycemic effect of Belamcanda chinensis leaf extract in normal and STZ-induced diabetic rats and its potential active faction. Phytomedicine. 2011 Feb 15; 18(4): 292-7.
67. Ajiboye BO, Ojo OA, Oyinloye BE, Okesola MA, Oluwatosin A, Boligon AA, Kappo AP. Investigation of the in vitro antioxidant potential of polyphenolic-rich extract of Artocarpus heterophyllus lam stem bark and its antidiabetic activity in streptozotocin-induced diabetic rats. Journal of evidence-based integrative medicine. 2020 May 15; 25: 2515690X20916123.
68. Jarvill-Taylor KJ, Anderson RA, Graves DJ. A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. Journal of the American College of Nutrition. 2001 Aug 1; 20(4): 327-36.
69. Maddux BA, See W, Lawrence Jr JC, Goldfine AL, Goldfine ID, Evans JL. Protection against oxidative stress—induced insulin resistance in rat L6 muscle cells by micromolar concentrations of α-lipoic acid. Diabetes. 2001 Feb 1; 50(2): 404-10.
70. Fröde TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. Journal of ethnopharmacology. 2008 Jan 17; 115(2): 173-83.
71. Robertson RP, Zhang HJ, Pyzdrowski KL, Walseth TF. Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. The Journal of clinical investigation. 1992 Aug 1; 90(2): 320-5.
72. Gulati V, Gulati P, Harding IH, Palombo EA. Exploring the anti-diabetic potential of Australian Aboriginal and Indian Ayurvedic plant extracts using cell-based assays. BMC complementary and alternative medicine. 2015 Dec; 15: 1-1.
73. Cui Z, Chen X, Lu B, Park SK, Xu T, Xie Z, Xue P, Hou J, Hang H, Yates III JR, Yang F. Preliminary quantitative profile of differential protein expression between rat L6 myoblasts and myotubes by stable isotope labeling with amino acids in cell culture. Proteomics. 2009 Mar; 9(5): 1274-92.
74. Kozma L, Baltensperger K, Klarlund J, Porras A, Santos E, Czech MP. The ras signaling pathway mimics insulin action on glucose transporter translocation. Proceedings of the National Academy of Sciences. 1993 May 15; 90(10): 4460-4.
75. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-γ2 transcription factor and TGF-β/BMP signaling pathways. Aging cell. 2004 Dec; 3(6): 379-89.
76. Gowri M. Gestational Diabetes Mellitus–Prevention by Life Style Modification. International Journal of Advances in Nursing Management. 2013 Sep 28; 2(3): 177-9.
77. Rojo LE, Ribnicky D, Logendra S, Poulev A, Rojas-Silva P, Kuhn P, Dorn R, Grace MH, Lila MA, Raskin I. In vitro and in vivo anti-diabetic effects of anthocyanins from Maqui Berry (Aristotelia chilensis). Food chemistry. 2012 Mar 15; 131(2): 387-96.
78. Vishnu PP, Srinivasa RA. Evaluation of anticancer activity of Tridax procumbens leaf extracts on A549 and Hep G2 cell lines. Asian J Pharm Clin Res. 2015; 8(3): 129-32.
79. Affourtit C, Brand MD. Stronger control of ATP/ADP by proton leak in pancreatic β-cells than skeletal muscle mitochondria. Biochemical Journal. 2006 Jan 1; 393(1): 151-9.
80. Dilger JP, Liu Y. Desensitization of acetylcholine receptors in BC3H-1 cells. Pflügers Archiv. 1992 Apr; 420: 479-85.

81. Cheng JH, She H, Han YP, Wang J, Xiong S, Asahina K, Tsukamoto H. Wnt antagonism inhibits hepatic stellate cell activation and liver fibrosis. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2008 Jan; 294(1): G39-49.
82. Castilho-Fernandes A, de Almeida DC, Fontes AM, Melo FU, Picanço-Castro V, Freitas MC, Orellana MD, Palma PV, Hackett PB, Friedman SL, Covas DT. Human hepatic stellate cell line (LX-2) exhibits characteristics of bone marrow-derived mesenchymal stem cells. Experimental and molecular pathology. 2011 Dec 1; 91(3): 664-72.
83. Kelly KA, Tanaka S, Baron R, Gimble JM. Murine bone marrow stromally derived BMS2 adipocytes support differentiation and function of osteoclast-like cells in vitro. Endocrinology. 1998 Apr 1; 139(4): 2092-101.
84. Begum MS, Aruna A, Sivakumar T, Premanand C, Sribhuaneswari MC. Invitro Cytotoxic Activity On Ethanolic Extracts Of Leaves Of Ipomoea Pes-Tigridis (Convolulaceae) Against Liver Hepg2 Cellline. International Journal of Ayurvedic and herbal Medicine. 2015 Jun 24; 5(3): 1778-84.
85. Qiu GH, Xie X, Xu F, Shi X, Wang Y, Deng L. Distinctive pharmacological differences between liver cancer cell lines HepG2 and Hep3B. Cytotechnology. 2015 Jan; 67: 1-2.
86. Taubman MB, Smith CW, Izumo S, Grant JW, Endo T, Andreadis A, Nadal-Ginard B. The expression of sarcomeric muscle-specific contractile protein genes in BC3H1 cells: BC3H1 cells resemble skeletal myoblasts that are defective for commitment to terminal differentiation. The Journal of cell biology. 1989 May 1; 108(5): 1799-806.
87. de Raemy-Schenk AM, Troublé S, Gaillard P, Page P, Gotteland JP, Scheer A, Lang P, Yeow K. A cellular assay for measuring the modulation of glucose production in H4IIE cells. Assay and drug development technologies. 2006 Oct 1; 4(5): 525-33.