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In Vivo and In Vitro Approaches to Anti-Diabetic Drug Screening

Prasanna N. Dalvi*, Azhar C. Shaikh, Hemant J. Pagar, Pandurang M. Gaikwad.

Department of Pharmacology, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad Ghat, Ahmednagar, India. - 414111

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KEYWORDS

Diabetes, Streptozotocin, Alloxan, Glucose uptake, Diabetes screening methods.

ABSTRACT:

Introduction: Diabetes mellitus is a prevalent medical disorder that can lead to severe complications. It is a significant global issue, and it is essential to induce the syndrome in animal models to further our understanding of its development. This understanding may lead to the development of novel treatments and, ultimately, a cure for diabetes. Approximately 90% of individuals with diabetes globally are diagnosed with type 2 diabetes. Several approaches are used to screen new antidiabetic medicines, such as in vivo and in vitro techniques. In vivo models commonly utilize chemicals like streptozotocin and alloxan to produce diabetes, whereas in vitro techniques demonstrate the direct impact of medicines on the cells involved in human diabetes. In-vitro approaches offer precise data and potential insights into the mechanisms behind diabetes development. Researchers have presented recent methods, such as using viruses to induce diabetes, which are proving beneficial for assessing antidiabetic medications. This review provides a comprehensive collection of information on diabetic models in a single location, which could be a valuable resource for researchers investigating diabetes.

Objectives: This review provides a comprehensive list of in vivo models and in vitro techniques relevant to diabetes research, aiming to aid researchers in making informed decisions and achieving significant outcomes in prevention, management, or treatment.

Conclusions: This review covers in-vivo and in-vitro models for researchers studying diabetes. Animal models have similar characteristics to human diabetes, providing essential tools for understanding endocrine physiology, metabolic changes, and genetic factors. Newer in vitro techniques are needed for evaluating and treating diabetes, while animal models and software-based studies are essential for advancement in diabetes research.

1. Introduction

Diabetes mellitus (DM) is a non-communicable chronic metabolic condition that is growing rapidly and posing clinical problems globally. It is defined by elevated amounts of glucose and lipids in the bloodstream, together with oxidative damage. These variables lead to long-term issues that impact different organs, such as the kidneys, eyes, nerves, and blood vessels. According to the World Health Organization (WHO), [1] The worldwide diabetes outbreak is a substantial and increasing public health issue. Approximately 537 million adults globally have diabetes, which accounts for one-tenth of the population. Experts predict that the number will rise to 643 million by 2030 and 783 million by 2045. The disease particularly impacts low- and middle-income nations, where almost three-quarters of individuals with diabetes live. Diabetes is a major contributor to death, causing approximately 6.7 million fatalities in 2021. The data highlights the immediate requirement for comprehensive programs that target the prevention, diagnosis, and efficient treatment of diabetes to reduce its severe effects on individuals, families, and healthcare systems worldwide. [2] Clinical symptoms and precise etiological characteristics categorize diabetes. There are two types of diabetes:

1. Diabetes associated with insulin deficiency (Type I, insulin-dependent, IDDM; 5 to 10% of all cases)

2. Diabetes associated with insulin resistance (Type II, non-insulin-dependent, NIDDM; 90 to 95% of all cases).

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Other types of diabetes include gestational diabetes, impaired glucose tolerance, and diabetes caused by other conditions or syndromes. [3]

Animal models that replicate the physiological and pathological alterations specific to each subtype of diabetes are critical for gaining a deeper understanding of this intricate illness and for proposing potential therapies. Researchers can choose and combine specific etiological causes and genetic backgrounds to create a specific type of experimental diabetes, allowing them to investigate specific biochemical or anatomical changes. Researchers can use animal models to explore disruptions observed in human diabetes. [4]

Examining diabetes using in vivo and in vitro methods is essential for gaining a deeper comprehension of the disease's pathology and development, and for uncovering novel treatments. Animal models are particularly beneficial in biomedical research for gaining significant insights into diabetes. [5]

Most animal models used in diabetes research are rodent-based. Rodents have shorter generation intervals, a small stature, and are cost-effective. Researchers can use various experimental techniques, including genetic alteration, chemical induction, and surgical operations, to induce diabetes in animals. We must design an adequate animal model and environmental conditions to evaluate new medications for treating diabetes. [6]

The objective of this review is to compile a thorough list of in vivo models and in vitro techniques that are pertinent to diabetes research. This review aims to be a beneficial resource for researchers conducting experimental work on diabetes. It provides a succinct and comprehensive guide that presents several options for consideration. The review aims to help researchers make well-informed decisions and attain significant outcomes, whether their focus is on the prevention, management, or treatment of diabetes.

2. Materials and Methods:

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

Streptozotocin-induced diabetes

Streptozotocin (STZ) is a toxic substance that damages insulin-producing beta cells in mammals and is used in chemotherapy for cancer treatments. It is an antibiotic effective against Gram-negative bacteria and is used in animal models of type 1 diabetes. STZ is considered carcinogenic, mutagenic, and potentially teratogenic by humans. [7] STZ is widely used by researchers to induce diabetes in animal experiments for creating models. STZ is widely used by researchers to induce diabetes in animal experiments for creating models. [8] Rats are given a high dose of STZ in type 1 diabetes models, or a low dose, in type 2 diabetes models, along with a high-fat diet, low STZ dosages, or in combination. [9,10]

Procedure:

We studied two groups of 84 male Wistar rats: a type 1 model and a type 2 model.

Type 1 Model: We divided 42 male Wistar rats into two groups: a sham control group with 10 rats and a diabetes group with 32 rats. Type 1 diabetes mellitus was induced by administering a high dose of 50 mg/kg of streptozotocin as a single dose while under ketamine anesthesia. Rats with plasma glucose levels exceeding 16 mmol/L on the third day post-STZ injection were subsequently assigned randomly to various groups. We obtained blood samples from the proximal ventral tail vein using a glucometer. We kept weekly records for fasting plasma glucose levels, water intake, and body weights, and assessed other parameters at the start and conclusion of the experiment. [11]

Type 2 model: Rats were given a high-fat diet for 8 weeks, followed by a low dose of STZ (25 mg/kg) over five days under ketamine anesthesia to induce type 2 diabetes. Rats with plasma glucose levels exceeding 16 mmol/L ten days post-STZ injection were randomly selected and allocated into several groups. We measured glucose levels by extracting whole blood from the proximal ventral tail vein using a glucometer. We took weekly measurements for fasting plasma glucose levels, water consumption, and body weights, and examined other parameters at the end of the experiment. [11]

The study terminated rats with cervical dislocation and obtained blood samples through cardiac puncture. The invasive assay evaluated protein expression indicators

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linked to CAN and diabetes problems, establishing a correlation with these markers approved by the CAN subcommittee. [12-16]

Evaluation parameters:

We anesthetized all rats with ether before taking blood samples from the retro-orbital plexus. We then allowed these samples to clot for an hour at room temperature. The separated serum was analyzed for various biochemical parameters, including serum ALT [17], AST [18], and ALP [19] activities, as well as creatinine [20] and urea [21]. We measured each parameter using a specific spectrophotometric diagnostic kit. Additionally, the glucose level in the urine of the rats was measured using Benedict's qualitative test.

Histological preparation: The liver was preserved in a solution of neutral buffered formalin (10%) and then dehydrated in a series of ethanol solutions with increasing concentrations (50%, 70%, 80%, 90%, 95%, and 100%). Afterwards, the tissue was embedded in Paraplast, sliced into thin sections of approximately 4 µm thickness, and finally stained with hematoxylin and eosin (H&E). We examined the resulting tissues under a light microscope for further analysis.

Alloxan induced diabetes

Alloxan (2,4,5,6-tetraoxypyrimidine; five,6-dioxyuracil) is a pyrimidine derivative synthesized by way of uric acid oxidation. it's far a hydrophilic volatile compound with a structure just like glucose. in the meantime, when alloxan is injected into an experimental animal, it generates a complex blood glucose response, along with changes in plasma insulin levels and structural alterations in beta cells. The alterations ultimately result in the demise of the beta cells. [22]

Procedure:

Following alloxan injection, the initial phase is a brief hypoglycemia period lasting for up to 30 minutes. [23,24] A hypoglycemia reaction causes a temporary rise in insulin secretion, confirmed by increased plasma insulin levels. Alloxan delivery increases blood glucose concentration, while plasma insulin levels drop during the initial hyperglycemic phase following pancreatic beta cell exposure. [23,25,26] The hyperglycemic phase lasts 2-4 hours and is characterized by reduced plasma insulin levels.

The changes in the body following an alloxan injection are caused by pancreatic beta cells suppressing insulin release. The injection induces toxicity in beta cells, resulting in this effect. The hypoglycemia phase, the third stage, begins 4–8 hours after the injection and persists for several hours.[23] During this phase, there is an increase in insulin circulation caused by the alloxan-induced rupture of secretory granules and cell membranes. [27,28]This leads to significant transitory hypoglycemia. Furthermore, additional subcellular organelles like the cisternae of rough endoplasmic reticulum, the golgi complex, and the outer and inner membranes of the mitochondria also experience ruptures. [28,29] Administering alloxan leads to permanent alterations in the pancreatic islets, indicating cell death. The fourth phase of the blood glucose response occurs 24-48 hours after injecting alloxan and is the final step of diabetic hyperglycemia. The beta cells undergo complete degranulation and lose their structural integrity in this phase. Non-beta cells, other endocrine and non-endocrine islet cell types, and additional pancreatic tissue are preserved, indicating the specific harmful effects of alloxan. Thus, alloxan injection causes a type I-like diabetes state that is dependent on insulin. All the morphological characteristics of beta cell destruction exhibit traits of necrotic cell death. [23,26,28,30]

Evaluation parameters: A study assessed fasting blood glucose (FBG) levels and body weight in 28-day-old animals, identifying induction restrictions through analysis of blood glucose levels.[31]

Monosodium glutamate-induced diabetic mice

Monosodium glutamate (MSG) is a commonly used food seasoning that is derived from glutamic acid. In the 1970s, studies were conducted on neonatal experimental animals to investigate the effects of injecting MSG. It is now widely known that neonatal treatment of adult mice and rats with MSG can result in the development of obesity along with other endocrine and metabolic abnormalities.[32]

Procedure:

The newborn mice were divided into two groups according to their treatments. The MSG mice received

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four daily subcutaneous injections of MSG (2 mg/g body wt) starting from their birth day, while the Saline mice received physiological saline injections. The study only included male mice, who were then divided into four groups at 4 weeks of age. Groups 1 and 2 of MSG mice, consisting of 12 males and 6 males respectively, received four weekly intraperitoneal injections of AOM (15 mg/kg body wt) and physiological saline (0.1 ml/10 g body wt) to form the MSG-AOM and MSG-Saline mice groups. Similarly, two groups of ICR-Saline mice were administered either AOM or saline. The sets were identified as groups 3 (consisting of 11 male Saline-AOM mice) and group 4 (comprising 5 male Saline-Saline mice). The experiment ended 10 weeks after the final AOM injection when the mice were 17 weeks old, and all animals were killed. The aim was to assess the quantity of colonic ACF and BCAC, perform a clinical serum chemistry test, and investigate the mRNA levels of IGF1, IGF2, and IGF-1R in the colonic mucosa.[33]

Dithizone induced diabetes

Dithizone is a sulfur and carbon chemical utilized to induce diabetes in animal models for research. Dithizone, when given to animals, leads to elevated concentrations of zinc, iron, and potassium in the bloodstream. Dithizone infiltrates the cells and combines with zinc, causing the release of protons and ultimately triggering the onset of diabetes.[34]

Procedure:

Administering a single intravenous dose of 40-100 mg/kg of different chelators like dithizone, 8-(p-toluenesulfonylamino)-quinoline, and 8-(benzenesulfanylamino)-quinoline can cause type 2 diabetes in cats, rabbits, golden hamsters, and mice. Dithizone injection induces a triphasic glycemic response in rabbits. Hyperglycemia is first observed after 2 hours, followed by a period of normal blood sugar levels after 8 hours, and then a sustained phase of high blood sugar after 24-72 hours. Microscopically, both complete and partial degranulation of beta cells can be seen.[35]

Monosodium glutamate induced diabetes

MSG elevates glutamate levels in the bloodstream. Administering MSG to mice leads to obesity and elevated insulin levels by stimulating insulin secretion. It also leads to elevated blood glucose, total cholesterol, and triglyceride level. [36,37]

Procedure:

Adult male Wistar rats are housed in a controlled laboratory setting with a temperature maintained at 25±3°C, humidity at 60±15%, and a 12-hour dark/light cycle. The rats are provided with regular rat chow pellets and filtered drinking water, with the option of including MSG based on their taste.

80 rats are randomly allocated into four groups, each containing 20 rats, to investigate the impact of MSG on rats. The groups are monitored for varying durations of 1, 3, 6, or 9 months, with each group consisting of 10 control rats and 10 rats treated with MSG. The rats treated with MSG get a 99% pure food-grade packet of MSG added to their daily drinking water at a final daily dose of 2 mg per gram of body weight.

Rats' food consumption and body weight are monitored at one- or two-week intervals. Rats from various groups are euthanized at 1, 3, 6, or 9 months after a 12-hour fast using intraperitoneal Nembutal injection. Blood and pancreatic tissue are gathered for analysis of their function and structure.[38]

Insulin antibodies induced diabetes

Antibodies that bind to insulin exhibit a higher affinity for it. Insufficient insulin function can cause postprandial hyperglycemia. Insulin bound to antibodies is unavailable to tissues, causing prolonged postprandial hyperinsulinemia and perhaps leading to hyperglycemia.[39]

Procedure:

Bovine insulin is dissolved in acidic water and then combined with a water-oil emulsion containing adjuvant. Male guinea pigs weighing 300-400 grams receive a monthly subcutaneous injection of 1 mg insulin, divided into doses. The guinea pigs are then blood sampled via heart puncture two weeks following the second and successive doses of antigen. 10 ml of blood can be extracted from each animal monthly.

Administering rats with guinea pig antinsulin serum intravenously, at dosages ranging from 0.25 to 1.0 ml, results in a blood glucose elevation that is depending on

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the dose. This rise can reach values up to 300 milligrams per deciliter. The effect observed is specific to guinea pig anti-insulin serum and happens when insulin antibodies neutralize the naturally produced insulin in the injected animal. This process results in insulin shortage as long as antibodies that can interact with insulin are present in the bloodstream. Administering high doses over an extended period through infusion or injection can lead to the presence of ketones in the blood and urine, as well as glucose in the urine, and can cause acidosis, potentially resulting in fatality for animals. Nevertheless, administering the substance by a slow intravenous infusion or intraperitoneal injection can extend the duration of the action beyond a few hours. At smaller doses, the diabetic condition can be reversed within a few hours. [4,40]

Goldthioglucose obese diabetic mouse model

Gold thioglucose (GTG) is a chemical capable of causing diabetes and obesity in animals. Administering GTG intraperitoneally can result in the progressive onset of obesity, hyperinsulinemia, and hyperglycemia. The substance is carried to cells, where it can induce necrotic lesions that promote the development of hyperphagia and obesity. Furthermore, GTG enhances hepatic lipogenesis, body lipid accumulation, and triglyceride production.[44]

Procedure:

Male and female Swiss albino mice are provided with unlimited access to commercial mouse chow. At six weeks of age, the mice get a single injection of 30-40 mg/kg Gold thioglucose into their peritoneal cavity. Food consumption of mice is monitored for two weeks, while their body weight is tracked over a three-month period. Subsequently, this data is compared with a cohort of untreated control mice to identify any disparities.[35]

Virus Induced Diabetes

Viruses cause destruction and infection of pancreatic beta cells, leading to the development of diabetes. Various human viruses such as Mengo-2T, reovirus, Coxackie B4, RNA picornaviruses, encephalomyocarditis (EMC-D and M types), and lymphocytic choriomeningitis have been associated with diabetes. [45,46]

Coxsackie viruses The Coxsackie virus in mice is recognized for its ability to damage pancreatic acinar cells, perhaps resulting in diabetes. The Coxsackie B4 virus is specifically associated with the onset of insulin-dependent diabetes mellitus in humans. Diabetes can be triggered by a Coxsackie virus infection leading to the release of stored islet antigen, which activates self-reactive T lymphocytes. [46,47]

In-vitro methods

Alpha amylase inhibition assay

To conduct experiment, 600 µl of test sample with concentrations ranging from 10µg/ml to 100µg/ml are combined with 1.2 ml of starch in a phosphate buffer solution. The solution has a pH of 6.9 and contains 6.7mM of sodium chloride. Next, combine the mixture with 600 µl of porcine pancreatic amylase and incubate at 37ºC. After incubation, 600 µl of the mixture is mixed with 300 µl of DNSA solution. The DNSA solution consists of 1g of DNSA, 30g of sodium potassium tartrate, and 20 mL of 2N sodium hydroxide, diluted to a final volume of 100 mL with distilled water. Afterward, the mixture is placed in a boiling water bath for 15 minutes. The reaction mixture is diluted with 2.7 ml of water, and the absorbance of the resulting solution is measured at 540 nm. Empty tubes are created for each concentration by replacing the enzyme solution with 600 µL of distilled water. A control tube is also produced to demonstrate 100% enzyme activity. The control tube is produced in the same way as the test sample, but without adding any test sample. The experiment is replicated thrice following the identical technique.[48]

The formula for assessment of Alpha- amylase inhibition percentage,

$$\%\ \alpha \text{ amylase inhibition} = \frac{\text{absorbance (control)} - \text{absorbance (sample)}}{\text{(absorbance (control)}} \times 100$$

α-glucosidase inhibition assay

Method A

Dissolve 0.5 mg of alpha-glycosidase in 10 ml of pH 7.0 phosphate buffer with 20 mg of bovine serum albumin to create an enzyme solution. Prepare this solution by mixing it with phosphate buffer at a ratio of 1:10. Dissolve 4 mg of the sample in 400 µl of dimethyl

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sulfoxide (DMSO) to create the sample solution, also known as the sample blank. Create solutions with volumes of 50, 100, 150, 200, and 250 µl at different concentrations. Combine P-nitrophenyl-α-D-glucopyranoside with phosphate buffer (pH 7.0) in 5 µl of the sample solution and DMSO. The solutions are kept at 37°C for 15 minutes. After 15 minutes, introduce a solution of Na2 CO3 (1000 µl). Quantify the absorbance of the sample compared to the sample blank at 400nm utilizing a UV-visible spectrophotometer.[49]

Method B

200 µl of alpha-glucosidase enzyme solution was produced and pre-incubated with varying doses of the test and standard drug solutions for 5 minutes. Next, 200 µl of 37 mM sucrose was added to each test tube. The tubes were kept at 37°C for 30 minutes to facilitate enzymatic and drug activity. After a 30-minute incubation period, the tubes were taken out and subjected to heating at 100°C for 10 minutes. The liberated glucose was determined using the glucose oxidase peroxidase (GOD-POD) technique. The blank control was utilized to determine the results at a wavelength of 546 nm.[50]

The formula for assessment of Alpha- glusidase inhibition percentage,

% α glusidase inhibition=

$$\frac{(\text{Absorbance (Blank)} - \text{Absorbance (test / standard)})}{(\text{Absorbance (Blank)})} \text{x}100$$

Inhibition of DPP-4 (Dipeptidyl Peptidase-IV) activity:

DPP IV inhibitors are a class of drugs utilized in the management of type 2 diabetes mellitus. The inhibitors function by impeding the degradation of two crucial hormones (GIP and GLP-1) that control glucose levels in the body. Research has demonstrated that inhibiting DPP IV can lower glucagon levels, slow down stomach emptying, and stimulate insulin release. DPP IV inhibitors show promise as a medication for controlling type 2 diabetes. Two methods are available for conducting this assay: the colorimetric method and the fluorometric method.[51]

Procedure:

A colorimetric approach was employed to investigate the inhibitory effect of flavonoids on DPP-4 activity, following the procedure outlined by Parmar et al.(52). The test observed the liberation of p-nitroanilide by breaking down GPPN with DPP-4 at a wavelength of 405 nm. The assay involved dissolving DPP-4 (150 U/mL) in Tris-HCl (50 mM, pH 8.0) and pre-incubating it at 37 °C for 10 minutes with flavonoids (0–200 µM) dissolved in DMSO (final concentration of 9.1% (v/v)). 0.25 mM of GPPN was added, which was dissolved in Tris-HCl (50 mM, pH 8.0). Kinetic measurements commenced promptly upon the introduction of GPPN and were sustained for 60 minutes at 37 °C. An enzymatic reaction was monitored using a microplate reader to measure absorbance at 405 nm. The concentrations of DPP-4 and the tested flavonoids relate to levels measured prior to the introduction of the substrate. Upon further analysis, it was found that the amount of DMSO used did not affect the assay. The data are shown as the % inhibition of DPP-4 activity, determined by analyzing the slope of the enzymatic reaction occurring between 5 and 60 minutes. Positive controls utilized were Sitagliptin ranging from 0 to 0.8 µM and diprotin A ranging from 0 to 500 µM. The findings collected are from a minimum of three separate experiments.[52]

Fluorometric method: The inhibitory effect of drugs/extract on DPP-4 activity was studied using a fluorometric test following a procedure outlined by Morikawa et al [53]. The procedure entails observing the breakdown of a substrate called GP-AMC by DPP-4 to produce a fluorescent product known as 7-amino-4-methylcoumarin (AMC) with excitation wavelength at 360 nm and emission wavelength at 460 nm. The test was conducted on a 96-well microplate. DPP-4 enzyme at a dosage of 38 U mL−1 was mixed with test flavonoids ranging from 0 to 200 µM, dissolved in DMSO at a final concentration of 9.1% (v/v), in TrisHCl buffer (50 mM, pH 8.0) and pre-incubated at 37 °C for 10 minutes. Later, GP-AMC at a concentration of 0.125 mM, dissolved in Tris-HCl at a pH of 8.0 and a concentration of 50 mM, was introduced. Photoluminescence measurements began promptly with the introduction of GP-AMC and continued for 60 minutes at 37 °C. The enzymatic reaction was observed by a microplate reader by quantifying the fluorescence at excitation and emission wavelengths of 360 ± 40 and 460 ± 40 nm, respectively. The concentrations of DPP-4 and the tested flavonoids

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relate to the levels measured before to adding the substrate. The amount of DMSO used had no impact on the assay. The effects are quantified as the percentage inhibition of DPP-4 activity, determined by analyzing the slope of the enzymatic reaction occurring between 5 and 60 minutes. The positive controls utilized were Sitagliptin ranging from 0 to 0.8 µM and diprotin A ranging from 0 to 250 µM. The results are derived from a minimum of three separate experiments.[54]

$$\% \text{ Inhibition} = \frac{(\text{Initial Activity}) - (\text{Inhibitor})}{(\text{Initial Activity})} \text{x} 100$$

In-vitro studies on insulin secretion

Anti-diabetic medications can impact many processes involved in glucose metabolism, such as insulin secretion, glucose uptake by specific organs, and food absorption. Recent in vitro investigations have explored the potential of incretins and peroxisome proliferator-activated receptors (PPAR) as targets for contemporary therapy. Insulin receptors and glucose transporters have not been focused on for anti-diabetic treatment. Several published research have examined the use of natural compounds for this aim.[55,56]

Studies using insulin-secreting cell lines

Bioengineering has developed new methods for producing cultured cell lines that are better suited for studying insulin secretion and cell dysfunction causes. Commonly utilized insulin-producing cell lines include beta-TC, RIN, HIT, MIN6, and INS-1 cells. These cell lines are valuable for investigating the molecular mechanisms underlying cell function. They mostly secrete insulin, as well as minor quantities of glucagon and somatostatin. While these cell lines do not precisely mimic basic cell physiology, they remain extremely valuable tools.[57]

Studies using isolated pancreatic islet cell lines

One can study the diabetes route by isolating pancreatic β-cells from healthy individuals or individuals with diabetes and culturing them in a suitable medium. Pancreatic cells utilize glucose to produce adenosine triphosphate (ATP) from adenosine diphosphate (ADP), leading to the production of insulin. This process entails a rise in the cytoplasmic ATP/ADP ratio, causing the closure of ATP-sensitive potassium channels. This results in the depolarization of the plasma membrane and the activation of voltage-dependent calcium channels. Consequently, this leads to an increase in the intracellular calcium concentration, which triggers the release of insulin. In type 2 diabetes, pancreatic cells display irregular ion channel behavior and an unusual insulin production pattern. These routes can be analyzed by isolating pancreatic cells from control or diabetic rats or mice using the collagenase digestion method, followed by separating and placing them in a suitable culture medium.[58,59]

In-vitro studies on glucose uptake

The primary connection between obesity and Type 2 diabetes is adipose tissue, which leads to lipotoxicity, causing cell damage due to high levels of intracellular lipids and insulin resistance. Insulin resistance at the adipocytes or skeletal muscle levels leads to hyperglycemia. Adipocytes in various body locations might have different physiologic or pathological impacts. Insulin resistance pathways can be investigated in adipocyte cell lines like marine 3T3-L1 cells and rat L6 muscle cells that have been modified to increase GLUT4 expression.[60,61]

Procedure:

1% suspension was created by dissolving baker's yeast from the market in distilled water. The suspension was left at room temperature (25°C) overnight. The following day, the yeast cell suspension underwent centrifugation at 4200 rpm for 5 minutes. The procedure was iterated by adding distilled water to the pallet until a clear supernatant was achieved. A 10% yeast suspension was prepared by mixing 10 parts of supernatant fluid with 90 parts of water. 1-5 mg of plant extract was dissolved in dimethyl sulfoxide (DMSO). The mixture was enriched with different concentrations (5, 10, and 25 mM) of 1 mL glucose solution and then incubated for 10 minutes at 37°C. Initiate the reaction by adding 100µL of yeast suspension to the combination of glucose and extract, vortexing, and incubating for 60 minutes at 37°C. Following incubation, the tubes underwent centrifugation at 3800 rpm for 5 minutes, and glucose levels were assessed with a spectrophotometer (UV 5100B) at a wavelength of 520 nm. The absorbance for the control sample was likewise measured at the same wavelength.[62]. The percent increase in uptake was calculated using the formula:

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% increase in glucose=$\frac{(\text{Abs.of control} - \text{Abs.of sample})}{\text{Abs.of control}}$x100

Glucose Adsorption Assay

The glucose adsorption assay is a technique utilized to quantify the glucose concentration in a sample. The process includes introducing the sample to a glucose solution with progressively higher concentration, stirring, and incubating it. After centrifugation, the sample's adsorption capability is directly related to the molar concentration of glucose.[63]

Procedure:

1 gram of extract was added to 100 mL of glucose solutions with concentrations of 5, 10, 15, 20, and 30 mM. The mixtures were thoroughly mixed, agitated, and then placed in a shaker water bath at 37°C for 6 hours. The mixture was centrifuged at 4800 revolutions per minute for 20 minutes after the incubation time. The glucose concentration in the supernatant was measured using a glucose oxidase peroxidase detection kit. (Ou et al., 2001)The amount of bound glucose was determined using a given formula:

Glucose bound = $\frac{G1-G6}{\text{weight of sample}} x$ volume of sample

Here, G1= glucose concentration of the original solution,

G6= glucose concentration after 6 hours.

donec.

3. Conclusion

This article aims to investigate the different in vivo models and in vitro techniques available for researchers to study diabetes. The in vitro models are categorized according to their significance in studying human diabetes. Each model offers crucial resources for studying the endocrine physiology, metabolic alterations, and genetic variations associated with the onset of diabetes in humans. Developing innovative in vitro approaches for diagnosing and treating diabetes is essential. Despite being costly, current in vitro techniques provide accurate pathways for studying the progression of diabetes. Additionally, it is necessary to create additional animal models and conduct software-based studies to progress diabetes research.

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