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Review Article

REVIEW ON HERBAL APPROACHES AND INVITRO AND INVIVO SCREENING METHODS OF ANTI-DIABETIC ACTIVITY

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Abstract:

Diabetes mellitus (DM), commonly known as just diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. Symptoms often include fatigue, frequent urination, increased thirst and increased appetite. If left untreated, diabetes can cause many health complications. Acute complications can include diabetes ketoacidosis, hyperosmolar hyperglycaemic shock, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney damage, foot ulcers, damage to the nerves, damage to the eyes and cognitive impairment.

Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus: Insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes-Type-1 diabetes, non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes-Type-2 diabetes, Gestational diabetes.

In this review we discussed about the various in-vitro and animal models for the screening of anti-diabetic activity. These methods include chemical, genetic, surgical manipulations relevant to the human diabetics. A brief description of Chemical causes of diabetes, virus induced diabetes, hormone induced diabetes and various other methods along with in-vitro techniques are explained.

Keywords: *Fatigue, Ketoacidosis, Gestational Diabetes, Genetic Manipulations.*

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1. INTRODUCTION:

Diabetes mellitus is a group of physiological dysfunctions characterized by hyper-glycemia resulting directly from insulin resistance, inadequate insulin secretion, or excessive glucagon secretion. Type-1 diabetes (T1D) is an autoimmune disorder leading to the destruction of pancreatic beta-cells. Type-2 diabetes (T2D), which is much more common, is primarily a problem of progressively impaired glucose regulation due to a combination of dysfunctional pancreatic beta cells and insulin resistance. Type-2 diabetes mellitus (DM) is a chronic metabolic disorder in which prevalence has been increasing steadily all over the world. As a result of this trend, it is fast becoming an epidemic in some countries of the world with the number of people affected expected to double in the next decade due to increase in ageing population, thereby adding to the already existing burden for healthcare providers, especially in poorly developed countries.

As of 2019, an estimated 463 million people had diabetes worldwide (8.8% of the adult population), with type 2 diabetes making up about 90% of the cases. Rates are similar in women and men. Trends suggest that rates will continue to rise. Diabetes at least doubles a person's risk of early death. In 2019, diabetes resulted in approximately 4.2 million deaths. It is the 7th leading cause of death globally. The global economic cost of diabetes-related health expenditure in 2017 was estimated at US\$727 billion. In the United States, diabetes cost nearly US\$327 billion in 2017. Average medical expenditures among people with diabetes are about 2.3 times higher. So, the demand for the discovery of the new drugs are much needed. The newly discovered drugs are to tested before hand in in-vitro and animal models before they are marketed.

1. Experimental studies of diabetes in animal models and advanced in vitro techniques are essential for the improvement of knowledge and clear understanding of the pathology and pathogenesis, and to find new therapy. Animal models of diabetes are therefore, greatly useful in biomedical studies because they offer the promise of new insights into human diabetes. Most of the available models are based on rodents because of their small size, shorter

generation intervals and economic considerations. Experimental diabetes mellitus studied by several methods that include: chemical, surgical and genetic manipulations. It is also very important to select appropriate animal model for the screening of new chemical entities (NCEs) and other therapeutic modalities for the treatment of diabetes. The main aim of the present review is to bring together all various in vivo animal models and in vitro techniques for carrying diabetes research.

HERBAL REMEDIES FOR DIABETES MELLITUS

Herbal medications have been used for the treatment of variety of ailments, a huge number of populations in the world is entirely dependent on traditional medicines. A number of medicinal plants and their formulations are used for treating diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices. In India, indigenous remedies have been used in the treatment of diabetes mellitus since the time of Charaka and Shusrutha. From the ethnobotanical information, about 800 plants which may possess anti-diabetic potential have been found. Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents. This practice may be due to its fewer side effects compare to the synthetic hypoglycemic agents and because of their safety, effectiveness, and availability. Although various synthetic drugs were developed to treat diabetes but still veryless number of drugs is available for the treatment of diabetes. There are about 200 pure compounds from plant sources reported to show blood glucose lowering effect. The compounds may be alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoids, peptides and amino acids, lipids, phenolics, glycopeptides and iridoids. Many antidiabetic products of herbal origin are now available in the market. More than 1200 species of plants have been screened for activity on the basis of ethnomedicinal uses. Here all the enlisted plants were pharmacologically tested in the alloxan induced diabetic rat's model system.

Table 1: Plants that possess Anti-Diabetic activity

S. NO.	SCIENTIFIC NAME OF PLANT	EXTRACT	ACTIVITY
1.	<i>Acacia arabica</i> (Leguminosae), <i>Benincasa hispida</i> fruit, <i>Tinispora cordifolia</i> stem, <i>Ocimum sanctum</i> (<i>O. sanctum</i>) areal parts and <i>Jatropha curcus</i> leaves.	The chloroform extracts of <i>A. arabica</i> bark in diabetic rats at 250 and 500 mg/kg, p.o. for two weeks. The other plant extracts also have similar effects.	Decreased the serum glucose level and restored total cholesterol (TC), triglyceride (TG), HDL and VDL levels.
2.	<i>Achyranthes rubrofusca</i> (Amaranthaceae) leaves.	The aqueous and ethanolic extracts of <i>A. rubrofusca</i> in diabetic rats.	It decreased the blood glucose level significantly, pancreatic enzyme such as superoxide dismutase (SOD), catalase (CAT) and glutathione level were significantly increased in the treated group compared to control group.
3.	<i>Andrographis paniculate</i> (Acanthaceae)	The oral administration of ethanol extract of <i>A. paniculate</i> in diabetic rats at a dose of 100 and 200 mg/kg, p.o. for 30 days treatment.	decreased the blood glucose level. Further it restored TG, TC, phospholipids, glycosylated haemoglobin, ALT, AST, ACP and ALP level which indicates its anti-diabetic activity.
4.	<i>Argyria cuneata</i> (Convolvulaceae)	The anti-diabetic activities of ethanol extract of leaves of <i>A. cuneata</i> in diabetic rats were investigated.	Anti-diabetic as well as lipid lowering potential.
5.	<i>Barleria prionitis</i> (Acanthaceae)	Alcoholic extracts of leaf and root of <i>B. prionitis</i> (Acanthaceae) in diabetic rats at 200 mg/kg, p.o. for 14 days treatment.	Decreased blood glucose and glycosylated haemoglobin level. Moreover, serum insulin and liver glycogen level were significantly increased.
6.	<i>Capparis decidua</i> (Capparaceae)	The aqueous and ethanolic extract of <i>C. decidua</i> stem in diabetic rats at 250 and 500 mg/kg, p.o. for 21 days treatment.	Decreased the blood glucose level which signified its anti-diabetic potential
7.	<i>Cassia grandis</i> (Leguminosae)	The aqueous and ethanolic extracts of <i>C. grandis</i> in diabetic rats at the dose level of 150 mg/ kg, p.o. for ten days treatment.	Decreased the blood glucose, TC, and TG level proving its anti-diabetic potential.
8.	<i>Colocasia esculenta</i> (Araceae)	Ethanol extract of <i>C. esculenta</i> in diabetic rats at 400 mg/kg, p.o. for 14 day, significantly	Decreased the blood glucose level and prevented loss of body weight. It indicates its anti-diabetic potential.
9.	<i>Costus igneus</i> (Costaceae)	Ethanolic extracts of leaves of <i>C. igneus</i> extracts in diabetic albino rats.	Reduction of blood glucose level and prevented body weight loss indicating its anti-diabetic potential.

10.	<i>Eucalyptus citriodora</i> (Myrtaceae)	Aqueous extract of <i>E. citriodora</i> leaf in diabetic rats at 250 and 500 mg/kg, p.o. for 21 days treatment.	Reduced the blood glucose level which confirms its anti-diabetic potential.
11.	<i>Ficus bengalensis</i> (Moraceae)	The aqueous extract of <i>F. bengalensis</i> bark in both IDDM and NIDDM rats at 1.25 g/kg, p.o. for 4 weeks.	Decreased the plasma glucose and serum lipids level.
12.	<i>Heinsia crinata</i> (Rubiaceae)	The ethanolic leaf extract of <i>H. crinata</i> in diabetic rats for 2 weeks.	reduced the fasting blood glucose levels. It indicates its anti-diabetic potential.
13.	<i>Helicteres isora</i> (Sterculiaceae)	The butanol and aqueous ethanol extracts of <i>H. isora</i> root in diabetic rats at 250 mg/kg for 10 days treatment was investigated.	Extract treated group showed decreased level of blood glucose, TC, TG and urea.
14.	<i>Ipomoea reniformis</i> (Convolvulus)	The ethanolic and aqueous extracts of stem of <i>I. reniformis</i> in diabetic rats at 300 and 600 mg/kg, p.o. for 12 days.	Anti-diabetic antihyperlipidemic potential.
15.	<i>Juglans regia</i> (Juglandaceae)	The methanolic extract of <i>J. regia</i> leaves was estimated in diabetic male wistar rats at 250 mg/kg and 500 mg/kg, p.o. for three weeks. <i>J. regia</i> significantly	Decreased the blood glucose, TG and TC level. Further it increased GPX, SOD and cell antibody level significantly and therefore signified its anti-diabetic potential.
16.	<i>Lantana aculeata</i> (verbenaceae)	The ethanolic extract of the dried mature roots of <i>L. aculeata</i> in diabetic rats at 25, 50 and 100 mg/kg, p.o. for 30 days treatment, was assessed.	Reduced the blood glucose level. Further it decreased TC and TG level and increased insulin and glycogen concentration in a dose-dependent manner, justifying its anti-diabetic potential.
17.	<i>Limonia acidissima</i> (Rutaceae)	Methanolic extract of <i>L. acidissima</i> in diabetic rats at 200 and 400 mg/kg, p.o. for 21 days treatment.	Decreased the blood glucose and MDA level. Further the activity of antioxidant enzymes such as SOD, CAT was found to be higher in treated group compared to the control group.
18.	<i>Luffa aegyptiaca</i> (Cucurbitaceae)	The alcoholic and aqueous extracts of <i>L. aegyptiaca</i> in diabetic rats at 100 mg/kg, p.o. for 15 days.	Decrease the blood glucose of hyperglycaemic rats which signifies its anti-diabetic potential.
19.	<i>Momordiccharantia</i> (Cucurbitaceae), <i>T. foenumgraecum</i>	Aqueous extracts of <i>M. charantia</i> pulp in diabetic rats for 30 days treatment was investigated.	Decreased the blood glucose levels. Moreover, all other parameter was significantly restored in the treated group compared to control group.
20.	<i>Mukai maderaspatana</i> (Cucurbitaceae)	The methanolic root extract	Decreased the blood

		of <i>M. maderaspatana</i> in diabetic rats at a dose of 500 mg/kg, p.o. for 21 days treatment.	glucose, TC, TG, LDL, and VLDL level. Further it decreased SGOT, SGP, ALP) and increased total protein (TP) content significantly at tested dose level.
21.	<i>Nymphaea pubescens</i> (Nymphaeaceae)	The ethanolic extract of <i>N. pubescens</i> in diabetic rats at 200 and 400 mg/kg, p.o. after 14 days treatment.	Histopathological examination of pancreas revealed its regenerative potential corroborating its anti-diabetic potential.
22.	<i>Ocimum gratissimum</i> (Lamiaceae), <i>Ocimum americanum</i> , <i>O. sanctum</i> and <i>Ocimum basilicum</i> .	The methanolic extracts of <i>Ocimum species</i> in diabetic Wistar rats at 500 mg/kg, p.o.	Reduction of blood glucose level with maximum potential in case of <i>O.sanctum</i> compared to the other tested extracts.
23.	<i>Paspalum scrobiculatum</i> (Poaceae)	Aqueous and ethanolic extracts of <i>P. scrobiculatum</i> in diabetic rats at 250 and 500 mg/kg, p.o. for 15 days treatment.	Reduced the blood glucose level and lipid parameters.
24.	<i>Phoenix dactylifera</i> (Arecaceae)	The <i>P. dactylifera</i> leaf extract in diabetes Wistar rats at 100, 200, and 400 mg/kg, p.o. and its fractions at 50, 100, and 200 mg/kg, p.o. for 14 days treatment.	Reduced blood glucose, TC, TG level and water intake but increased plasma insulin level significantly compare to control group.
25.	<i>Phyllanthus niruri</i> (Euphorbiaceae)	The methanol extract of aerial parts of <i>P. niruri</i> in diabetic rats significantly	Reduced the blood glucose, TC and TG in a dose-related manner.
26.	<i>Phyllanthus simplex</i> (Euphorbiaceae)	Various fractions of <i>P. simplex</i> such as petroleum ether (200 and 400 mg/kg), ethyl acetate (100 and 200 mg/kg), methanol (125 and 250 mg/kg), water fraction (150 and 300 mg/kg).	Anti-diabetic and the active fractions also restored the antioxidant enzymes levels in liver and kidney.
27.	<i>Pongamia pinnata</i> (Fabaceae)	The standardized ethanolic extract of <i>P. pinnata</i> in diabetic rats was tested for its anti-diabetic potential.	After 21-day treatment it was found that <i>P. pinnata</i> possess significant anti-diabetic activity.
28.	<i>Solanum nigrum</i> (Solanaceae)	Aqueous leaf extracts of <i>S. nigrum</i> in diabetic rats at 200, 400 mg/kg b.w. for 21 days.	Reduced the blood glucose and other lipid parameter.
29.	<i>Sphenostylis stenocarpa</i> (Leguminosae)	The methanolic extract of seeds of <i>S. stenocarpa</i> in diabetic rats at the doses of 200, 400 and 600 mg/kg.	Reduced the blood glucose level. Moreover, 600 mg/kg, p.o. was found to be more significant compared to other tested dose level.
30.	<i>Tephrosia villosa</i> (Fabaceae)	Ethanolic extract of leaves of <i>T. villosa</i> in diabetic rats at two different doses, showed significant	Histopathological examination of pancreas showed regenerative power and therefore signified its

		reduction in the blood glucose level.	anti-diabetic potential.
31.	<i>Trigonella foenum-graecum</i> (Fabaceae)	Ethanol extract of <i>T. foenum-graecum</i> seeds in diabetic rats at 2 g/kg, 1 g/kg, 0.5 g/kg and 0.1 g/kg, p.o. was investigated.	Among all the tested dose level, 1 g/kg, p.o. was found to be more significant comparing to other dose levels.
32.	<i>Vaccinium arctostaphylos</i> (Ericaceae)	The ethanolic extract of <i>V. arctostaphylos</i> fruit in diabetic male rats for 3 weeks.	Decreased the blood glucose and triglyceride level. However, it increased the erythrocyte SOD, glutathione peroxidase, catalase activities and expression of GLUT-4 and INS genes.
33.	<i>Zaleya decandra</i> (Aizoaceae)	Effect of ethanolic extract of <i>Z. decandra</i> roots in diabetes rats at 200 mg/kg, p.o. for 15 days treatment.	Significantly restored the levels of glucose, TC, TG, TP, urea, creatinine, lipid peroxidation level, and antioxidant enzymes.
34.	<i>Zizyphus mauritiana</i> (Rhamnaceae)	The petroleum ether and aqueous extract of <i>Zizyphus mauritiana</i> (Rhamnaceae) at 200 and 400 mg/kg, p.o. doses.	Elevated biochemical parameters such as glucose, urea, creatinine, TC, TG, HDL, LDL, haemoglobin, and glycosylated haemoglobin.

2. CHEMICAL INDUCED DIABETES

2.1 Streptozotocin induced diabetes

Streptozotocin (STZ) is a naturally occurring chemical it particularly produces toxic to the beta cells of the pancreas. It is used in medical research as an animal model for hyperglycemia. STZ alters the blood insulin and glucose concentrations. Two hours after injection, the hyperglycemia is due to the decreased in blood insulin levels. Six hours later, hypoglycaemia occurs due to the high levels of blood insulin. At last hyperglycemia develops and blood insulin levels drops. STZ impairs glucose oxidation and decreases insulin synthesis and release. It was observed that STZ at first abolished the B cell response to glucose. STZ restricts GLUT2 expression. STZ changes the DNA in pancreatic B cells. The B cell death is due to alkylation of DNA by STZ. STZ-induced DNA damage activates poly ADP-riboseylation. The activation of poly ADP-riboseylation is of greater importance for the diabetogenicity of STZ than generation of free radicals and DNA damage. Calcium, which may also induce necrosis.

2.2 Alloxan induced diabetes

Alloxan is most widely used in experimental diabetic research. Alloxan produces selective necrosis of the

beta cells of pancreas. The alloxan is administered by various routes like intravenous, intraperitoneal and subcutaneous. Alloxan is used for induction of diabetes in experimental animals such as mice, rats, rabbits and dogs. The routes and dose of alloxan required may vary depending upon the animal species.

A First short lived hypoglycemic phase lasting for 30 min from the first minutes of alloxan administration. The hypoglycemic stage may be due to the stimulation of insulin release and high levels of plasma insulin levels. The mechanism at back of the hyperinsulinemia is due to the short-term increase of ATP availability and glucokinase inhibition. The second phase is the increase in the blood glucose levels one hour after administration of alloxan, the plasma insulin concentration decreases. The pronounced hyperglycemia lasts for 2-4 hours is due to decrease plasma insulin concentrations. This may be due to inhibition of insulin secretion and beta cell toxicity. The third phase is hypoglycemic phase that long last for 4-8 hrs after administration of alloxan.

Alloxan treatment brings out a sudden rise in insulin secretion in the presence and absence of glucose. The insulin release occurs until the complete suppression

of the islet response to glucose. Alloxan react with two sulfhydryl in the glucokinase resulting in disulphide bond and inactivation of the enzyme. The alloxan is reduced by GSH. Superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous ions. Fe^{3+} can also be reduced by alloxan radicals. Another mechanism reported is the fragmentation of DNA in the beta cells exposed to alloxan. The disruption in intracellular calcium levels also contribute for the diabetogenic action of alloxan.

2.3 Monosodium glutamate induced diabetes

Monosodium glutamate (MSG) cause increase in plasma glutamate concentration. MSG stimulates insulin release. Administration of MSG in mice resulted in obesity associated with hyperinsulinemia. After 29 weeks level of blood glucose, total cholesterol and triglyceride levels were increased.

2.4 Dithizone induced diabetes

Dithizone is an organosulfur compound, it has chelating property. Dithizone is used in induction of diabetes in experimental animals. In dithizonised diabetic animals, the levels of zinc, iron, and potassium in the blood were found to be higher than normal. Dithizone has permeates membranes and complex zinc inside liposomes, then release of protons, this enhances diabetogenicity.

2.5 Insulin antibodies induced diabetes

The insulin antibodies have the affinity and capacity to bind insulin. Insulin deficiency mechanism may cause greater postprandial hyperglycemia because antibody-bound insulin is unavailable to tissues, but the prolongation of postprandial hyperinsulinemia may lead to hyperglycemia.

2.6 Goldthioglucose obese diabetic mouse model

Gold thioglucose (GTG) is a diabetogenic compound, which manifest obesity induced Type -2 diabetes. The intraperitoneal administration GTG in experimental animal gradually develops obesity, hyperinsulinemia, hyperglycemia, insulin resistance for a period of 16- 20 weeks. The GTG is transported in particular to the cells and causes necrotic lesions, which is responsible for the development of hyperphagia and obesity. It also increases body lipid, hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreases glucose metabolism.

2.7 Ferric nitrilotriacetate induction of diabetes

In experimental animals' parenteral administration on of large daily dose of ferric nitrilotriacetate for 60 days manifest diabetic symptoms such as hyperglycemia, glycosuria, ketonemia and ketonuria.

3. VIRUS INDUCED DIABETES

Viruses produce diabetes mellitus by destroying and infecting pancreatic beta cells. Various human viruses used for inducing diabetes include RNA picornoviruses, Coxsackie B4, encephalomyocarditis (EMC-D and M variants), Mengo-2T, reovirus, and lymphocytic choriomeningitis.

3.1 Coxsackie viruses

Coxsackie viruses also cause diabetes in mice; it can infect and destroy pancreatic acinar cells. Coxsackie B4 virus is strongly associated with the development of insulin-dependent diabetes mellitus in humans. Diabetes induced by Coxsackie virus infection release of sequestered islet antigen resulting in the re-stimulation of auto reactive T cells.

3.2 D-Variant Encephalomyocarditis (EMC-D)

EMC- D virus can infect and destroy pancreatic beta cells in mice and produce insulin dependent hyperglycemia. EMC-D virus known as NDK25. Intraperitoneal injection of NDK25 develops non-insulin dependent diabetes mellitus.

4. HORMONE INDUCED DIABETES

4.1 Corticosteroid induced diabetes

Corticosteroid induces diabetes, which is called steroid diabetes. The prednisolone and dexamethasone, cause steroid diabetes. Glucocorticoids stimulate gluconeogenesis, in the liver, resulting in increase in hepatic glucose and induce insulin resistance and hyperglycemia.

4.2 Growth hormone induced diabetes

Repeated administration of growth hormone in higher experimental animals induces diabetes with ketonuria and ketonemia. Prolonged administration of growth hormone produced permanent diabetes; there was loss of pancreatic islets tissues and of beta cells.

5. SPONTANEOUS DIABETIC OBESE RODENT MODELS

5.1 db/db mouse

The db gene mutation occurs spontaneously in the leptin-receptordeficient C57BL/KsJ mice and is originally derived from mutation on chromosome 4. The db/db mouse becomes hyperphagic, hyperinsulinemia, and insulin resistant within 2 weeks of age, obesity at the age of 3 to 4 weeks. The hyperglycemia develops at the age of 4 to 8 weeks. At this age, the mouse exhibits ketosis and body weight loss occur. The db/db mouse was used to study renal and micro vascular diabetic complications.

5.2 Ob/ob mouse

The ob/ob mouse strain, have leptin deficiency because of the mutation in leptin gene leading to severe insulin resistance. The ob/ob mice exhibit rapid gain in body weight, insulin resistance and hyperinsulinemia occur. In the ob/ob model, hyperinsulinemia manifests at 3 to 4 weeks of age together with hyperphagia and insulin resistance. The symptom of Type-2 DM of ob/ob mice attenuates with age, continuous decline of plasma insulin levels in the second year of life, glucose tolerance and insulin resistance.

5.3 Tsumura Suzuki Obese Diabetes (TSOD) mouse

TSOD mouse, exhibits obesity and insulin resistant at 2 months old, which contributes for hyperinsulinemia and hyperglycemia. In TSOD mouse, pancreatic islets are hypertrophic. The impaired GLUT4 translocation in both skeletal muscle and adipocytes of TSOD mouse causes reduced insulin sensitivity and insulin resistance.

5.4 New Zealand Obese (NZO) mouse

The New Zealand strain of obese mice, gains weight at 10 weeks of life as a result of hyperphagia, hyperglycemia and hyperinsulinemia. NZO mouse manifests insulin resistance at an early age. With the growth of NZO mouse, hyperglycemia and glucose tolerance increase and the level of blood glucose reaches 300-400mg/dL at the age of 20 to 24 weeks. It is useful model for studying obesity and diabetes.

5.5 Kuo Kondo mouse

The Kuo Kondo (KK) mouse is model of obesity and Type-2DM. It has been crossed with the Bar Harbor C57BL/6J mouse. KK mouse spontaneously exhibits distinct adiposity, hyperglycemia, and hyperinsulinemia. At 2 months of age, the KK mouse manifested obesity due to hyperphagic, insulin resistance and compensatory hyperinsulinemia. The insulin resistance and hyperinsulinemia reached to the peak at 5 months.

5.6 Zucker Diabetic Fatty (ZDF) rat

The Zucker diabetic fatty (ZDF) rats are less obese, more insulin resistant, and rapidly progress to diabetes due to lack of sufficient insulin secretion. The male ZDF rat becomes fully diabetic at 12 weeks. The serum insulin levels of male ZDF rat reach the peak at about 7 to 10 weeks, but cannot respond to glucose stimulus and the insulin levels drops.

5.7 Otsuka Long-Evans Tokushima Fatty (OLETF) rat

The OLETF rat develops hyperglycemia at around 18 to 25 weeks age. OLETF rats exhibits obesity, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia, and onset of diabetes similar to human Type-2DM. Many recessive genes on several chromosomes including the X chromosome are involved in the induction of diabetes in OLETF rats.

5.8 M16 mouse

M16 mice manifest obesity at all ages due to hyperphagia. At 8 weeks of age, all M16 mice exhibit hyperglycemia, hyperinsulinemia, and hypercholesterolemia.

5.9 Nagoya-Shibata-Yasuda (NSY) mouse

The NSY mouse, imitates human Type-2DM with the characteristics are mild obesity, impaired insulin secretion and insulin resistance contributing to diabetes development in an age dependent manner. NSY mice, all males develop diabetes, while females is only about 30%. The NSY mouse is particularly useful for studying the age-related damages and phenotypes of Type-2 DM.

6. SPONTANEOUS DIABETIC NON-OBESE RODENT MODELS

6.1 Spontaneously Diabetic Torii (SDT) rat

SDT rat is a new spontaneously non-obese diabetic strain. It has characteristics like glucose intolerance, hyperglycemia, hyperinsulinemia, and hypertriglyceridemia. Because of the severe hyperglycemia, SDT rats develop diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy. This model is suitable for studying complications of human T2DM.

6.2 Cohen diabetic rat

Cohen diabetic rat is a genetic model derived from diet-induced Type-2 DM model by placing the rat on a synthetic 72% sucrose-copper-poor diet for 2 months, manifest the human Type-2 DM. The manifestations include non-obesity, hyperinsulinemia, and insulin resistance. The Cohen diabetic rat expresses genetic susceptibility to a carbohydrate-rich diet, a feature of Type-2 DM in human.

6.3 Goto-Kakizaki (GK) rat

The GK rat is a non-obese model of T2DM with hyperglycemia, hyperinsulinemia, and insulin resistance. In GK rats a stable fasting hyperglycemia was observed at the end of the first 2 weeks. After 8 weeks, hyperglycemia degenerates and insulin secretion of the islets stimulated by glucose. GK rats, develops complications of diabetes like peripheral neuropathy, and retinopathy.

6.4 Surgical Model of Diabetes Mellitus

Surgery technique used to induce diabetes, is complete removal of the pancreas. Limitation to this technique include high level of technical expertise and adequate surgical room environment. Pancreatectomy has been employed; large resection is required to obtain mild to moderate hyperglycemia.

7. IN VITRO TECHNIQUES

7.1 In-vitro studies on insulin secretion and glucose uptake

The oral antidiabetic agents can affect several pathways of glucose metabolism such as insulin secretion, glucose uptake by target organs as well as nutrient absorption. Incretins and transcription factors such as peroxisome proliferator activated receptors-PPAR are targets of modern therapy. Insulin receptor, glucose transporters, has not been focused for antidiabetic therapy.

Adipose tissue is considered to have a link between obesity and Type-2 diabetes, elevated intracellular lipid concentrations and insulin resistance. Insulin resistance either at the adipocyte or skeletal muscle levels contribute to hyperglycemia. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as marine 3T3-L1 cells and rat L6 muscle engineered to over-express GLUT4.

7.2 Inhibition of α -glucosidase activity

The α -glucosidase enzyme inhibition activity was performed by incubating α -glucosidase enzyme solution with phosphate buffer which contains test samples of different connections at 37°C for 1 hr in maltose solution. The reaction mixture was kept in boiling water few min and cooled. Glucose reagent was added and its absorbance was measured at 540 nm to estimate the amount of liberated glucose from maltose by the action of α -glucosidase enzyme. The percentage of inhibition and IC50 was calculated.

7.3 Assay of amylase inhibition

In vitro amylase inhibition can be studied by adding the test sample was allowed to react with α - amylase enzyme and incubated, add starch solution. After incubation dinitrosalicylic acid reagent was added to both control and test. Keep this mixture in boiling water bath for few minutes. The absorbance was taken at 540 nm using spectrophotometer and the percentage of inhibition of α -amylase enzyme was calculated.

A starch solution was prepared with potato in sodium phosphate buffer, sodium chloride and kept in a boiling water batch for few min. The α -amylase

solution was prepared by mixing α -amylase in the same buffer. The colorimetric reagent was prepared by mixing equal volume of sodium potassium tartrate tetra hydrate solution and 3,5-dinitro salicylic acid (DNS) solution. Starch solution was mixed with test sample with various concentration or acarbose and α -amylase solution was added and incubated at 25°C to react with the starch solution. DNS reagent was added to the above solution, and the contents were heated for 15min on a boiling water bath. The final volume was made up with distilled water, and the absorbance was measured at 540nm using spectrophotometer. The percentage inhibition and IC50 value was calculated.

7.4 Studies using isolated pancreatic islet cell lines

These pathways can be studied with isolated pancreatic cells from experimental animals that can be obtained by collagenase digestion technique, followed by adequate separation and transference to appropriated culture medium. It is known that insulin secretion occurs when pancreatic cells utilize glucose to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage dependent Ca^{2+} channels. This results in elevation of the intracellular Ca^{2+} concentration which triggers insulin secretion.

8. CONCLUSION:

As diabetes mellitus is a common disorder which the whole world is facing. So, the research and discovery of new medicines and various treatment techniques need to be developed much. In this review we discussed about the available animal models and in vitro techniques for the screening of anti-diabetic activity. The discussed models are most appropriate for the testing because they have similar features and characteristics to humans. Each model is essentials tools for investigating endocrine, metabolic, genetic changes and underlying mechanism of human diabetes. The animal models and in vitro techniques are essentials for developing a new drug for the treatment diabetes. Now a days many software, advanced techniques are developed that help a lot in diabetic research.

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