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A COMPREHENSIVE REVIEW ON *IN VITRO* AND *IN VIVO* MODELS USED FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

Diabetes mellitus- commonly known as diabetes, is a disease characterized by high blood sugar level. This high blood sugar level is due to either the body does not produce insulin in normal level or cells do not respond to the insulin produced by the body. According to World Health Organization(WHO), diabetes is currently one of the biggest health concerns that the world is faced with. WHO defines diabetes as "a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces". According to statistics of IDF(International Diabetes Federation), India has more diabetics than any other countries of the world. As per the current statistics the number of diabetics in the country is ranging about more than 62 million and it is expected by the year of 2030, over 100 million people in India are likely to suffer from diabetes. The current review paper highlights about the various *in vivo* and*in vitro* models that are used to evaluate this alarming disease.

KEYWORDS: Diabetes mellitus, Insulin, *in vitro*, *in vivo* and models.

INTRODUCTION

Pancreas is a gastro intestinal, endocrine organ lied in the upper abdomen behind the stomach.Itsecretes digestive enzymes in to the intestine, hormones into the blood stream to control energy metabolism. Pancreas comprises of two parts-a. exocrine pancreas comprises more than 95% of the pancreas, it includes acinar, duct cells associated with connective tissues, vessels and nerves. This portion of pancreas produces and secretes enzymes that are

helpful for digestion. b. Endocrine pancreas - also known as “the islet”, comprises 1-2 % of the whole pancreatic mass. It produce and secrete insulin, glucagon, somatostatins and pancreatic polypeptide hormonesinto the blood. [1] Insulin is a polypeptide hormone produced by the β cell of pancreatic islet of langerhans. It maintains glucose level in blood by cellular glucose uptake, carbohydrate regulation, lipid metabolism and protein metabolism. It also promote celldivision and growth through mitogenic effects. [2]

Insulin deficiencies

a. **Insulin resistance:** It is a condition of normal or increased insulin level resulting in different biological responses.[2&3]
b. **Compensatory hyperinsulinaemic:**It is a condition resulting from the increased beta cell secretion to maintain normal blood glucose level resulting from the peripheral insulin resistance in muscle and adipose tissue.[2]
c. **Diabetes mellitus:**Commonly known as diabetes, is a disease characterized by high blood sugar leveland shows characteristic symptoms like polydipsia (increased thirst), polyuria (frequent urination) and polyphagia (increased hunger). The high blood sugar level is due to either the body does not produce insulin in normal level or cells do not respond to the insulin produced by the body.Diabetes can be divided to different types according to the cause of disease.[4]

Type 1 diabetes: It is also named as insulin dependent diabetes mellitus. It is characterized by insulin deficiency resulting from beta cell necrosis due to invasion by virus, action of chemicals or action of autoimmune anti-bodies.[5&6]

Type 2 diabetes: It is also named as non-insulin dependent diabetes mellitus and it ischaracterized by target organ insulin resistance, which resulting responsiveness to both endogenous and exogenous insulin.[5&7]

Type 3 diabetes: Type 3 diabetes is a proposed form of Alzheimer’s disease, characterized by decreased utilization of glucose and resulting insulin resistance in brain. Patients with alzheimer‟s shows less insulin and insulin receptors than the normal subjects and treatment with insulin has been associated with improved memory and cognition.[8-10] This type of diabetes is also obtained by chronic pancreatitis or chronic treatment with glucocorticoids, thiazide diuretics and with some protease inhibitors.[5]

Type 4 diabetes: This type of diabetes is normally seen in pregnancy duration due to placental hormones that promotes insulin resistance.[5&11]

Pathogenesis of diabetes mellitus

The auto immune elimination ofbeta cell which is mediated by factors such as environmental and viral are responsible for IDDM. The beta cell destruction leads to decreased insulin release and obesity leads to insulin resistance which is responsible for NIDDM.[12-14]The metabolic impairment of glucose in skeletal muscle and liver is mediated by insulin resistance. Due to this resistance glucose intolerance and hepatocyte destruction will takes place' which mediated to hyperinsulinaemia by theincreased level of growth hormone, glucagon, free fatty acids and cytokines. All the above factors indirectly result in dysfunction of beta cell of pancreas which finally result in diabetes mellitus.[12&16]

Diagnosis of diabetes

Measurement of blood glucose: If a personhaving plasma glucose greater than 7.0mmol/l (126 mg/dl) or 2 h plasma glucose greater than 11.1mmol/l (200mg/dl) then we can say that person is suffering from diabetes. Measurement of blood glucose level is the diagnostic criteria for diabetes, glucose can be measured by separating plasma from blood immediately or blood is collected into a tube containing glycolytic inhibitors and place on ice water until separated prior to analysis.[17]

Oral glucose tolerance test (OGTT): The oral glucose tolerance test (OGTT) measures the time it takes for glucose to exit fromyour blood after absorbing a glucose drink. The test will take approximately 2.5 hours (Half an hour rest prior to test and two hours test time).Results of the OGTT not only diagnose diabetes but can decide if you have weakened fasting glucose (IFG) or weakened glucose tolerance (IGT). Having either of these conditions indicates a significantly increased risk of developing diabetes in future.[18]

Glycated Haemoglobin (HbA1c): Now a day's HbA1c is not considered as a suitable conclusion test for diabetes due to its cut point as 6.5 % in blood. If the value is less than 6.5 % other glucose tests are performed to confirm diabetes.[17&19]

Fasting blood glucose test: Blood glucose can checked after fasting in the middle of 12 and 14 hours. Patients should strictly avoid any other beverage, allowed to take water during this

time and diabetic patients advised to detain their diabetes treatment or insulin dose until the test is completed.[17&20]

Random blood glucose test: Blood glucose levels are measured randomly in a day, the test doesn't affect the time of eating. Constant test result indicate the person is free from diabetes.[17&20]

MODELS TO EVALUATE ANTI DIABETIC PROPERTIES OF DRUGS

I. *IN VIVO* ANIMAL MODELS OF DIABETES MELLITUS

a) Chemically induced diabetic animals

i. Alloxan induced diabetes

Alloxan is a urea imitative used to induction of type 1 diabetes in animals like rabbits, rats, mice and dogs. Diabetes is generated by the selective destruction of beta cell of pancreatic islet.[5&21]

Mechanism of action

Alloxan administration makes a sudden rice in insulin secretion in the existence or absence of glucose for a short duration followed by result in the complete suppression ofthe islet reaction to glucose even when large concentrations of glucose isused.[5, 22&23]

Method

Diabetes is induced by intraperitoneal injection of 150mg/kg of freshly prepared alloxan monohydrate in normal saline to overnight fasted rats. After 72h of injection rats with blood glucose levels more than 200mg/kg were considered diabetic and selected for the study.The blood glucose levels and body weight were measured on day 1, 7 and 14 of the study.[24]

ii. Streptozotocin (STZ)

STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is cytotoxic especially to β-cells of the pancreas, it is utilized to produce IDDM in animal model and NIDDM with multiple low doses. It is also used in medicine for treating metastatic cancer of islets of Langerhans.[5&25]

Mechanism of Action

In mammalian cells streptozotocin acts by the prevention of DNA (Deoxyribonucleic acid) synthesis, via entering to the pancreatic cell by the help of glucose transporter-GLUT2 (Glucose transporter 2) and generate alkylation of DNA. Further STZ induces activation of

poly adenosine diphosphate ribosylation and nitric oxide release, as a result of nitric oxide release pancreatic -cells are destroyed by necrosis and finally induced insulin dependent diabetes.[5,26 &27]

Method

Diabetes was generated by single intraperitoneal injection of Streptozotocin (45 mg/kg b.w) dissolved in freshly prepared 0.1M of cold citrate buffer, administered to overnight fasted rats. Rats with fasting blood glucose concentration, >250 mg/dl considered as diabetic and selected for further experimentation. During the study period, blood glucose levels and body weight of all the rats were determined at regular meantime. At the end of the study, the rats were overnight fasted, anaesthetized, and sacrificed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation respectively and analyzed for sugar levels.[28]

iii. Dithizone

Dithizone administration shows increased level of serum zinc, iron and potassium than normal animals but copper and magnesium levels were unchanged. Most of these serum levels were normal after treatment with insulin, except for serum potassium and magnesium.[5&29]

Mechanism of Action

Dithizone permeate the membranes and form a complex with zinc present inside the liposomes with the liberation of protons, which can enhance diabetogenicity. Such proton release occurs within the zinc-containing insulin storage granules of pancreatic beta-cells; the solubilisation of insulin would be occur which mediate to osmotic stress and eventually the granule break and finally diabetes is induced.[5&30]

Method

Diabetes induction was obtained by intraperitoneal administration of Dithizone (5mg/kg body weight) to the rats. They were allowed for 72h for development of diabetes. After 72 h, rats with blood glucose levels more than 200mg/kg were considered diabetic and selected for the study. Blood was drawn for biological estimations after 06, 12, 18 and 24hrs of oral administration test drug.[31]

iv. Monosodium glutamate

Monosodium glutamate producestype -2 diabetes without polyphagia. It will increase plasma glutamate concentration and stimulates insulin secretion.[5, 12& 32]

Mechanism of Action

Monosodium glutamate causes glycosuria in mice without polyphagia. The glucose concentration in blood, total cholesterol and triglyceride were higher within 29 weeks.[5&33]

b) Hormone Induced Diabetes

Some hormones also modify insulin release in response to glucose. The islet of Langerhans comprises four types of cells i.e β, α, δ and Polypeptide (pancreatic polypeptide) cells. The β-cell produces insulin, which is a potent anabolic hormone having a growth promoting effect and multiple synthetic effect. The α-cell secrete glucagon, which induce hyperglycemia by its glycogenolytic activity in liver. The δ-cell produce somatostatins, which suppress both insulin and glucagon release and polypeptide cells produce a unique pancreatic polypeptide that makes several effects like pancreatic enzyme secretion and inhibition of gall bladder contraction.[12&34]The repeated dosing of growth hormone in rats do not show any sign of diabetes, but grow faster, the similar treatment in adult dogs and cats make intensive diabetes with all signs including severe ketonuria and ketonemia.[17&35]

i. Role of growth hormone in diabetes mellitus

Growth hormone is one of glucose counter regulatory hormone. Rising in level of growth hormone lead to insulin resistance and hyperglycemia.[12&36]

ii. Role of Corticosteroid in diabetes mellitus

Corticosteroid is utilized to reduce inflammation but it can lead to diabetes. Glucocorticoids dislike insulin action and invigorating gluconeogenesis, mainly in the liver, resulting in a net increase in hepatic glucose output and induce insulin resistance, hyperglycemia and hyperlipidemia which is called steroid diabetes. Prednisolone and dexamethasone are the most frequent glucocorticoids which generate steroid diabetes.[5&37]In rat's steroid diabetes is generated withcortisone which is secreted from adrenal cortex by the stimulation of corticotrophin.[17&38]

c) Viruses Induced Diabetes

Viruses can induce diabetes in two ways such as either direct (infection) destruction of beta cells or by initiation of an autoimmune response to beta cells.[12,17&39]

i. D- Variant Encephalomyocarditis

"M variant" of EMC virus induce diabetes by initiation of diabetic like syndrome and it selectively infect pancreatic cells. In certain inbred strains of mice EMC- D virus can infect and demolish pancreatic beta cells and produce insulin dependent hyperglycemia. In ICR Swiss mice pre-treatment with a potent immunosuppressive drug, cyclosporine-A expand acuteness and occurrence of diabetes.[5,40&41]

ii. Coxsackie Viruses

Coxsackie viruses can cause diabetes in mice by infection and demolition of pancreatic acinar cells while leaving the adjacent islets of Langerhans intact. In humans Coxsackie B4 virus is strongly related with the evolution of insulin-dependent diabetes mellitus. Diabetes generated by Coxsackie virus infection is a direct outcome of local infection resulting in inflammation, tissue damage and re-stimulation of resting auto reactive T cells resulting from the release of sequestered islet antigen, further indicating that the islet antigen sensitization is an indirect outcome of the viral infection.[5, 42&43]

d) Genetically diabetic animals

Genetically or Spontaneously diabetic animals aregenerally inherited diabetes either by single or multi gene defects.Single gene defect (monogenic) may be obtained by dominant gene (*e.g.*, Yellow obese or KK/Ay mouse) or recessive gene (diabetic or db/db mouse, Zucker fatty rat) or it can be of polygenic origin [*e.g.*, Kuo Kondo (KK) mouse New Zealand obese (NZO) mouse]. Genetically diabetic animals of NIDDM may be obtained from the animals with one or several genetic mutations transmitted from generation to generation (e.g., ob/ob,db/db mice)or by selected from non-diabetic outbred animals by repeated breeding over several generation [e.g.,(GK) rat, Tsumara Suzuki Obese Diabetes (TSOD)mouse].[44&45]

I. Spontaneously diabetic rats

1. Bio Breeding (BB) Rat

The BB rat is a spontaneous diabetes model, which is related with insulin deficiency and insulitis due to autoimmune demolition of pancreatic beta cells by immune attack with T cells, B cells. The immunosuppressive agent mycophenolate mofetil can avert the evolution of diabetes in BB rats. The onset of clinical diabetes generally seen at 60-120 days of age and

severe hyperglycemia continue after several days related with hypoinsulinemia and ketosis.[12, 17 &46]

2. Cohen diabetic rat(CDR)

Cohen reported that hyperglycemia, glucosuria and hyperinsulinemia are characteristics of Cohen rats. It's a derived model of diet induced type 2 diabetes. Itshows genetic susceptibility (sensitivity and resistance) to a carbohydrate-rich diet.[12,17,44,47&48] Cohen rats is noticed with a late evolution of β- cell dysfunctioning, hypoinsulinemia, insulin resistance and a lessen in the number and sensitivity of insulin receptors.[12,17&48] The Cohen rat strain, metabolic phenotypes of the rebred colony of CDs (Cohen diabetic sensitive) and CDr (Cohendiabetic resistant) rats are useful experimental model that is mostly suitable for studying the interaction between nutritional-metabolic environmental factors and genetic susceptibility for the progress of type2 diabetes and also useful for investigating the effect of sex on the expression of diabetic phenotype.[44&48]

3. WBN/KOB rat

The animals of Wistar strain also named as WBN/KOB rat are associated with impaired glucose tolerance and glucosuria at 21 weeks of age. The degeneration takes place mainly around islets and pancreatic ducts at 16 weeks old male rat, they also develop demyelination, predominantly motor neuropathy.After 12 weeks of agefibrinous exudation and deterioration of pancreatic tissue in the exocrine part and lessen in the number and size of islets is observed.[47&49]

4. Goto-Kakizaki (GK) rat

GK (Goto-Kakizaki) rats are non-obese polygenic model of NIDDMwith highly inherited strain of Wistar rats that spontaneously develop type II diabetes. It is established by Goto and his collaborators by selective inbreeding of Wistar rats with abnormal glucose tolerance repeated over several generations in Japan in 1973.[17, 44 &50] GK rat is mainly used for studying the relation of changes in beta cell mass, occurrence of NIDDM and diabetic complications (particularly diabetic nephropathy).[44] Diabetic complications are specified by non-obesity, average but stable hyperglycaemia in adult, hypoinsulinaemia, normolipidaemia, impaired glucose tolerance, glucose stimulated insulin secretion, peripheral insulin resistance along with impaired skeletal muscle glycogen synthase activation by insulin.It is also accompanied by chronic activation of diacylglycerol-sensitive protein kinase C .Which are all appears at 2-4 week of age.[12, 17, 44, 50-52]

5. Zucker-fatty rat

Zucker-fatty rat is a classic model of hyperinsulinemic obesity. It is specified by hyperphagia and early onset of obesity (which appear at 4 week of age) due to a simple autosomal recessive (fa) gene, along with increased growth of subcutaneous fat deposition. It also shows mild hyperglycaemia, peripheral insulin resistance similar to human NIDDM, mild glucose intolerance, hyperlipidaemia, hyperinsulinaemia and moderate hypertension.[17,44,53-56] Different insulin sensitizing and anti-obesity agents are screened by using Zucker fatty rat.[12&57]

6. Zucker diabetic fatty rat (ZDF/DRT-FA)

It is a substrain of ZFR, selectively inbred for hyperglycaemia and useful for the investigation of mechanism related with insulin resistance and β- cell dysfunction in type 2 diabetes.[12&44] It is less obese but more insulin resistant than ZFR, extreme hyperphagia due to the loss of calories by glucosuria are the characteristics of these animals. In these diabetes is produced by lipotoxicity to the beta-cell.[17, 44, 58&59]

7. WDF/TA-FA

Wistar Kyoto rats are developed by shifting of the fatty (fa) gene from the Zucker rat, which exhibits genetically obese, hyperinsulinemia, hyperlipidemia, hyperphagia and more insulin resistant.In normal female rats hyperglycemia is not seen but it can be generated by addition of sucrose to the diet.[17&60]

8. OLETF rat

OLETF (Otsuka Long Evans Tokushima Fatty) rats with polyuria, polydipsia and mild obesity was obtained from the selective breeding of spontaneous diabetic rats from the outbred colony of Long Evans rats maintainedin Otsuka pharmaceuticals, Tokushima, Japan in 1984. This model is widely used in pharmacological research like antidiabetic and antihypertensive drugs.[17, 44,61-63]In 1995 Aizawa et al., found that OLETF rats completely avert the evolution of obesity and insulin resistance from the age of 4 to 12 weeks. But, Insulin resistance preceded impaired insulin secretion in OLETF rats when diazoxide (0.2% in diet), an inhibitor of insulin secretion was administered. The common specific properties of OLETF rats are a chronic course of disease, renal complications (nodular lesions), mild obesity, hyperglycemia onset is late (after 18 weeksof age), hyperplastic foci of pancreatic islets,males inheritance, resembling of clinical and pathological properties with human

NIDDM features.[17&64]In OLETF rat's, diabetes is developed by defects in the beta cell proliferation.[44&65]

9. ESS-rat

ESS-rat is a colony of rats with the occurrence of spontaneous diabetes. Six months old rats shows the disarrangement of the islet anatomy and fibrosis of the stroma. From the age of 2 months onwards the animals show unusual glucose tolerance tests with a syndrome of a mild type of diabetes that does not affect the longevity of the animal.[17, 66&67]

10. OBESE SHR rat

Koletsky made the strain of obese SHR rats by mating a spontaneous hypertensive female rat of the Kyoto-Wistar strain with a normotensive Sprague- Dawley male. Russell et al., described that JCRLA-corpulent rat substrains of obese SHR rat's exhibits a syndrome characterized by obesity, hypertriglyceridemia and hyperinsulinemia.[17,68&69]Friedman et al., found that the obese spontaneously hypertensive Koletsky rat have a reduced insulin receptor signaling effect.[17&70]

11. JCR: LA (James C Russel-LA) -Corpulent rat

JCR: LA (James C Russel-LA) -Corpulent rat used as research model for development of atherosclerotic and myocardial lesions in association with syndrome- X. The major disadvantage of this rat is it become normoglycaemic when fasted.[44&71] The ultimate metabolic profile including insulin resistance, hyperinsulinaemia, pancreatic beta cell hyperplasia, obesity, glucose intolerance and severe hyperlipidaemia are exhibited because of the presence of recessive gene (*cp / cp*). The *cp* gene encodes a stop codon in the leptin receptor producing nonfunctional receptor protein. Incorpulent rats the leptin receptor deficient states along with hypothalamic dysregulation of peptides contribute to hyperphagia and other metabolic abnormalities.[44]

12. SHR/N-CP rat

Adamo et al., developed the congenic SHR/N-*cp* rat (spontaneously hypertensive rat/NIH-corpulent) by inbreeding of SHR/N strains, by mating a male Koletsky rat heterozygous for the corpulent gene (cp/+) to a female rat of the Okamoto strain at the National Institute of Health (NIH), Bathesda, Maryland, USA.It is a genetic model for investigating obesity, NIDDM with hypertension.[17,44&72] SHR/N-CP rat shows obesity, mild hypertension, hyperinsulinemia and glucose intolerance. It isalso used for studying the influence of dietary

carbohydrate on the development of diabetes in certain genetically predisposed carbohydrate sensitive individuals.[17&44]

13. BHE rat

The BHE rat's shows the diabetic state only at maturity. This rat colony was originally obtained by breeding black and white hooded rats of the Pennsylvania State College strain and albino rats of the Yale (Osborne Mendell) strain. These rats exhibit hyperinsulinemia at 50 days of age. Glucose intolerance and tissue resistance to insulin resulting in hyperglycemia.[17&73]

II. Spontaneously diabetic mice

1. KK mouse

KK (Kuo Kondo) mouse alsoknown as Japanese KK mouse, is a polygenic model of obesity and NIDDM developed by selective inbreeding in Japan.[44,73&74] These animals are characteristic with hyperphagic, hyperinsulinaemic, insulin resistant and show average obesity by insulin resistance at 2 months old, which remains maximum at 4-5 months. There is an elevation of pancreatic insulin content is seen along with increase in number and size of pancreatic islets but histologically degranulation of beta cells and hypertrophy of islets of pancreas are found, also there is a depletion of glycogen and an elevation in lipid content shown in sections of the livers.[12, 17, 44, 75- 77]

2. KK-AY mouse

KK-AY mouse of 5 weeks old shows increased levels of blood glucose and circulating insulin as well as hemoglobin A1c (HbA1c).[12,17&78] Histo and immunochemical studies show that islets of pancreasare hypertrophied, degranulated and glycogen infiltration of beta-cells, lipogenesis by liver and adipose tissue were increased.[17, 44, 76 &78]Diani et al., reported that the early onset and fast growth of glomerular basement membrane thickening is an indication of the renal involvement.[17&78] All These findings concluding that the principal reason for diabetes in KK-AY mice which carrying a yellow obese gene(AY) is insulin resistance which maybe because of defects in both insulin receptor and post receptor signaling systems, inclusive of glucose uptake, pentose pathways and impaired insulin sensitive phosphodiesterase in fat cells. These mice are utilized for investigation of the extra pancreatic action of antihyperglycemic drugs, such as glimepiride, a novel sulfonylurea.[17&44]

3. NOD mouse

The NOD mouse is a model of IDDM and develops insulitits at age 4-5 week old, followed by subclinical autoimmune destruction of β-cell. These strain was obtained by inbreeding diabetics. These mice derived originally from the JCLICR strain.[12, 17, 79 & 80]The pancreatic β-cells destruction is proceeded by dependent auto-immune process of CD4+ and CD8+ T-cell resulting in insulin-dependent diabetes mellitus and the use of a soluble interleukin-1 receptor or an immuno modulating drug can prevent the onset of diabetes in NOD mice.[17, 80-82]They usually die due to ketosis. If insulin treatment is not given the NOD mouse does not survive for more than one month.[17& 80]

4. OBESE hyperglycemic mice

The hypertrophy and hyperplasiaof pancreatic islets result in obesity (pear shaped body), that will form by diabetes like syndrome of hyperglycemia, slightly altered glucose tolerance, severe hyperinsulinaemia, sub fertility and impaired wound healing.[44&83]Bleisch et al., observed that there is a hereditary diabetes in genetically obese micewith characteristic features as glycosuric, insulinresistance, the non-fasting blood sugar levels are about 300mg%, but neither ketonuria nor coma is observed. Insulinresistance is the highlighting feature. The obese hyperglycemic mice is different from the diabetic condition of the human diabetic patient.[17, 84&85]

5. Diabetes mouse (DB/DB)

Diabetes mouse (DB/DB) is now relabeled as *leprdb* because of the autosomal recessive mutation of leptin receptor gene derived diabetes in db/db mouse.These mouse shows a severe diabetic symptom indicated by early onset of hyperinsulinaemia and hyperglycemia.[17, 44, 86 &87]The leptin receptor (Ob-R) gene encodes 5 different spliced forms.[17&88]These mice is not a completely type 2 diabetic model because mice shows pancreatic isletvolume and it is dramatically greater but some abnormalities in insulin secretion is seen, although islet maintains insulin secretion and lack of complete β-cell failure.[12]

6. Diabetes obesity syndrome in CBA/CA mice

Male CBA/Ca mice have a spontaneous maturity of onset diabetes obesity syndrome that occurs at 12-16 weeks old with a small proportion (10-20%), it can increase up to 80% after inbreeding. Diabetic obesity syndrome is indicated by hyperphagia, obesity, hyperglycemia,

hypertriglyceridemia and hyperinsulinemia. Female mice remains normal except with a slight elevation in serum insulin. Exogenous insulin is resistant to these mice.[17&89]

7. Chinese hamster

Chinese hamsters have high blood sugar levels as a normal of 110 mg% up to 600 mg%., which shows diabetic symptoms as severe polyuria, glucosuria, ketonuria and proteinuria. The treatment with insulin and oral antihyperglycemic drugs will improved the diabetic symptoms. The numbers of islets langerhans are reduced and the cells of the existing islets are abnormal.[17]

III. Other species with inherited diabetic symptoms

i. Sand rat

The sand rat (*Psammomys obesus*) lives in the desert regions of North Africa and near East, which is used as model of latent type 1 diabetes mellitus it develops obesity, diabetic symptoms within 2-3 months when fed chow(high energy diet) instead of an all-vegetable diet. Symptoms as hyperphagia, hyperinsulinaemia, glucose intolerance were seen. The pancreatic islet cells remain intact followed by beta cell deterioration and necrosis resulting in acute insulin insufficiency and over diabetes and ketosis ultimately leading to death of animal.[12, 17, 44, 90- 94]

ii. Spiny mouse

Spiny mouse (*Acomys cahirinus*) is a nocturnal that weight 30 -50g and having fur brister on their backs, large light brown mice which is seen in the semi-desert areas of the Eastern Mediterranean.[17,12&95] They are low insulin secretors but accumulate the insulin in beta cells, the disintegration of this insulin may produce insulin-deficiency. When they fed with on high energy rodent lab chow, they attain weight and shows marked pancreatic beta cell hyperplasia, hypertrophy and increased pancreatic insulin content. In comparison to other animal models, the impairment of the plasma insulin reaction to glucose as well as to other secretogogues is suggesting an impairment in hormone release mechanisms.[44, 73, 76&92]

iii. African hamster (*Mystromys albicaudatus*)

African hamster is a type for spontaneous diabetes mellitus, characterized by polyuria, polyphagia, polydipsia and pancreatic lesions which include β cell vacuolization, glycogen infiltration, nuclear pycnosis and margination of organelles, pancreatic lesion and β-cell death.[12, 17 &96]

iv. TUCO-TUCO

Tucotucos (*Ctenomis talarum*) shows a similar diabetic syndrome to *Psammomys obesus* rats and *Acomys cahirinus* mice. It is characterized by degranulation of β-cells, normal bruise in pancreas, but amyloid hyalinization of islets has been observed.[12,17 &97]

v. MACACA NIGRA (Celebes black apes)

Macaca nigra show anelevated occurrence of spontaneous diabetes mellitus with characteristic signs as hyperglycemia, decreased clearance of glucose, atherosclerosis with stiffened basement membranes of muscle capillaries. These shows less insulin secretion and elevated serum lipids.[17, 98&99]

e. Transgenic Animals

i. Transgenic Mice

Transgenic mouse is a prototype for chronic hyperglycemia. It is used for examine the role of genes and their effects on peripheral insulin action such as insulin receptor, IRS-1, IRS-2, glucose transporter (GLUT 4), peroxisome proliferator activated receptor-γ (PPAR-γ).[17,44 &100]

ii. Surgically induced diabetic model

a. Pancreatectomy in dogs

70 or 90 per cent of partial pancreatectomy dissection reported in various animal species mostly in dogs, pigs, rabbit and rats results inpolyuria, polydipsia, polyphagia and severe glucosuria. The injection of concentrate of the pancreatic glands shows depletion of the high blood sugar levels in pancreatectomized dogs.[17,44,83,101-103] Pancreatectomized dogs marked by average hyperglycaemia with neither reduction in body weight nor reduction in plasma insulin levels, insulin resistance. Improvement in insulin resistance is observed by administration of insulin or phlorizin which is an inhibitor of renal glucose reabsorption.[44, 104 &105]

f. Miscellaneous Models

i. Invertebrate animal model

In this model silk worm *Bombyx mori* is utilized for the assesment of anti-diabetic drugs. When the silk worm is fed with a high-glucose diet (10% glucosecontaining diet) for 3 days which elevates 4 fold hemolymph sugar level compared to silk worms fed on a normal diet.

The hemolymph sugar level elevates following intake of up to a 33% glucose diet, body size and weight was also used as other parameters for this model.[17]

ii. Diet Induced metabolic dysregulation

In this model male albino Wistar rats and non-human primates Baboon (*Papio hamadryas* sp.) are commonly used. The diabetes was generated in male albino wistar rats by a high fructose diet (66 % fructose and 1.1% coconut oil) resulting in increased glucose and glycosylated haemoglobin level.[12&106]In non-human primates Baboon (*Papio hamadryas* sp.) diabetes was produced with high sugar, high fat diet after 12h fasting. The composition of diet includes 73% Purina Monkey Chow 5038 (a grain-based meal), 7% lard, 4% Crisco, 4% coconut oil, 10.5% flavored more fructose corn syrup, and 1.5% water. After 8 weeks of dietary exposure non-human primates Baboon shows an expansion in body fat and triglyceride concentration is observed along with change in percentage glycosylated hemoglobin (HbA1c) and adipokines. The baboons and humans are similar in genetically, anatomically and physiologically, metabolic disorders.[17&107]

II. *INVITRO* MODELS FOR ANTIDIABETIC ACTIVITY ASSESSMENT

In vitro studies are utilized to determine specific mechanisms and toxicities, which are performed by biological materials such as perfused whole organs, isolated tissues, cell culture systems or tissue slice preparations. Antihyperglycemic effects can be examined by *invivo* animal models or *invitro* using a variety test methods such as inhibition of carbohydrate digesting enzyme model, inhibition of intestinal glucose uptake modeletc.,.[108]

a) Models to study inhibition of Carbohydrate digesting enzymes

i. Assay of α-amylase inhibitory activity

To 500µl of various concentrations of prepared test samples and standard drug (Acarbose)add 500µl of 0.20mM phosphate buffer (pH 6.9) holding α-amylase (0.5mg/ml) solution. The contents were then incubated at 25°C for 10min. To this 500µl of a 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was add on to each tube. The solution mixtures was then incubated at 25°C for 10min. The reaction was stopped with 1.0ml of 3, 5 dinitrosalicylic acid color reagent ((1 g 3,5-dinitrosalicylic acid in a solution containing 20mL of 2mol/L NaOH, 50mL distilled water and 30g Rochelle salt). The test tubes were then incubated in a boiling water bath for 5min, allow to cool at room temperature. The reaction content was then diluted after adding 10ml distilled water and absorbance was read at 540nm.50% Inhibitory Concentration (IC50) is calculated by the following formula

I % = (Ac-As)/Ac X 100

Where Ac is the absorbance of the control and As is the absorbance of the sample.[109-111]

ii. Assay of α-glucosidase inhibitory activity

Dahlqvist method is used to prepare crude enzyme solution of rat intestinal α- glucosidase and sucrose. The test was performed according to the method of Honda and Hara.[108,112 &113] Different concentrations of the sample is mixed with ten milliliters of enzyme solution and incubated for 10min at 37°C and the volume was made up to 210µl with maleate buffer pH 6. Additionof 200µl of 2mM p-nitrophenyl-α-D-glucopyranoside solution starts the enzymatic reaction and incubated at 37°C for 30min. The reaction is stopped by keeping the mixture in a boiling water bath for five minutes. Add 1ml of 0.1M disodium hydrogenphosphate solution, the absorption of liberated p-nitrophenol is measured at 400nm.[108]

iii. Assay of sucrase inhibitory activity

Different concentrations of the sample is mixed with ten milliliters of enzyme solution and incubated for 10min at 37°C and the volume was made up to 210µL with maleate buffer pH 6. Additionof 100µl sucrose solution (60mM) starts the enzyme reaction, keep it as such for 30 minutes and add 200µL of 3, 5- dinitrosalicylic acid reagent, keep it in a boiling water bath for terminating the reaction. Measure the absorbance of solution at 540nm

I % = (Ac-As)/Ac X 100

Where Ac is the absorbance of the control and As is the absorbance of the sample.[108]

b) Models to study inhibition of intestinal glucose uptake

The response of insulin and insulin-mimetic substances on muscle tissue are studied using stimulation of glucose uptake by the isolated diaphragm of mice and rats. The washed rat diaphragms are incubated (30 min, 37 °C) in HEPES-buffered saline (25mM HEPES, 120mM NaCl, 5mM KCl, 1.5mMCaCl2, 1mM MgCl2, 5mM glucose, 0.5 mM sodium pyruvate, 1.5mM KH2PO4, pH 7.4) with a constant bubbling of 95% O_2/5% CO_2. Then the diaphragms are washed to two times with the same buffer lacking glucose solution and further incubated (30min) in 5ml of glucose-free buffer in the existence of test compounds or insulin. The addition of 50ml of 10mM 2-[1- 3H] deoxyglucose (10 mCi/ml) in the lack or existence of 25mM cytochalasin B (control) will initiate the glucose transport. The diaphragms are rinsed four times with ice cold buffer comprising 10mM glucose, 25mM cytochalasin B after 15min of glucose transport, blotted with filter paper and homogenized the rinsed diaphragmportions of the suspension are utilize for protein determination.1ml

portions of the supernatant (centrifuged at 10000 *g* for 15min), mixed with 10ml scintillation cocktail and counted for radioactivity. One Specific glucose transport (dpm/mg of protein) is measured as the dissimilarity between diaphragm-related radioactivity measured in the lack (total uptake) and existence of cytochalasin B (non-specific uptake). Transport is linear for 30min under these studying conditions.[108&114]

c) Models to study insulin secretion from β cells of the pancreas

Cultured cell lines is used to facilitate studies of mechanisms of both insulin secretion and beta-cell dysfunction.The largest widely used insulin-secreting cell lines are RIN, HIT, beta-TC, MIN6 and INS-1 cells. Which release mainly insulin and small amounts of glucagon and somatostatins. Although the behavior of none of these cell lines perfectly mimics primary beta-cell physiology, also utilize for the investigation of molecular events underlying beta-cell function.[115&116]The**HIT cell line** is an insulin secreting cell line developed by Santerre et al.,1981 from the hamster. The isolated pancreatic islets, dispersing into single cells, transforming the cell isolates with the simian virus 40 (SV40) and the insulin secreting cell lines are obtained by cloning method.[108&117]Masuda et al., and Asfari et al., (1995) performed an experiments for assessment of glucose transport activity in HIT cells and Western blot analysis for GLUT2 in these cells after incubation with glibenclamide and troglitazone.[108&118]INS-1 cells and INS-2cells are derived from parental RINm5f cells. The betacyte, also named as HEP G2ins/g cell, is a genetically engineered insulin-secreting human hepatic cell line with a property as glucose responsive.[108,119-121]

d) Models based on muscle as an insulin target tissue

Obesity and type 2 diabetes are promoted by the development of lipotoxicity, here the key link between these two are adipose tissue i.e. cell damage as a consequence of elevated intracellular lipid concentrations and insulin resistance. Hyperglycemia is a result of insulin resistance either at the adipocyte or skeletal muscle.[108&122]

i. Total uptake of glucose

Adipocytes are incubated with D-[U-14C] glucose (0.2 mM final concentration) for 20min. Centrifugation on silicon oil separate the cells are from the medium, counted for radioactivity. The total insulin-stimulated glucose uptake (signal cascade, glucose transport and glucose metabolism) was measured by this assay, irrespective of whether the glucose is used via the oxidative or non-oxidative pathway. It also detects the transformation into lipids, glycogen or membrane-impermeable midway products (glucose-6-phosphate).The following

assay quantifies the whole glucose uptake into cells with inactivated insulin receptor. So the substance that bypass the first step in the insulin signal transduction cascade (binding of insulin to its receptor) will show positive results. Adipocytes are incubated with trypsin(4 mg/ml) for 15 min at 4 °C. The cells are washed three times by flotation after inclusion of soy bean trypsin inhibitor(8 mg/ml) and used for examination of total uptake of glucose.[108]

ii. Transport of 2-deoxy-glucose

The transport of 2-deoxy-D-[1-3H] glucose(Amersham, specific activity 20–30 Ci/mmol, aqueous solution) was measured by isolated rat adipocytes or murine 3T3-L1 cells and rat L6 muscle engineered to over-express GLUT4 described by Gliemann et al., (1972).[108,123 &124]

e) Models based on adipocytes as an insulin target tissue

In vitro techniques shows there an increased glucose uptake in muscle tissue and in adipocytes.[108, 125&126] HepG2 cells activate insulin receptors, which gives different variety of metabolic responses to insulin and insulin-like growth factor-1 via the substrate as cytoplasmatic protein insulin-receptor substrate-1 (IRS-1). In HepG2 and BC3H-1 muscle cells troglitazone (CS-045) have an elevated glycogen synthase I activity.IRS-1 undergoes multisite tyrosine phosphorylation and moderatedownstream signals by ‘docking’. Insulin derivatives can be indicated by phosphorylation and dephosphorylation kinetics of the insulin receptor, insulin receptor substrate-1 and which is incriminated in mitogenic signal transduction.[108&127]

CONCLUSIONS

The current updated reviewtries to cover the various *in vitro* and *in vivo* models that are used for screening the antidiabetic activity of a test drug. This updated review paper can bemuch useful for the researchers who are presently involved in the research and development of a new antidiabetic drug.

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