ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.7826]

Journal Homepage: www.eijppr.com

Review Article

A Review on Different Types of Animal Models for Pharmacological Evaluation of Antidiabetic Drugs

Sangeeta Saini, Savita Kumari, Santosh Kumar Verma, Anil Kumar Sharma*
CT Institute of Pharmaceutical Sciences, Shahpur, Jalandhar-144020 (Punjab), India.

Article info

Article History:
Received 15 May 2013
Accepted 7 June 2013

Keywords:
Diabetes mellitus, Pathogenesis, Animal model, Herbal pant, Biomarkers

Abstract

Diabetes mellitus is a disorder of carbohydrate, fat and protein metabolism which is characterized by absolute or relative lack of insulin resulting in hyperglycemia. This disease is remains to be an expanding global health crisis, because it is potentially morbid condition with high prevalence worldwide thus it constitutes a major health concern. Due to its high prevalence and deleterious effects, many animal models such as alloxan induced, streptozotocin induced, high fructose induced model and viruses induced etc have been developed for evaluating the antidiabetic activity of various agents. Synthetic drugs used to control the diabetes mainly oral hypoglycaemic but due to serious side effects of synthetic drugs herbal products is a better alternative without any serious side effects like hypoglycaemia to controlled diabetes mellitus so in this review we concluded that plants reported to prevent the diabetes induced by different models of diabetes having different pathogenesis and novel biomarkers may became a future prospective due to its long lasting and safer effects.

1. INTRODUCTION

Diabetes mellitus is a disorder of carbohydrate, fat and protein metabolism, which is characterized by absolute or relative lack of insulin, resulting in hyperglycemia[1]. It is mainly of two types: type 1 diabetes mellitus and type 2 diabetes mellitus. Long term diabetes mellitus leads to a variety of complications such as cardiovascular disease[2], retinopathy[3], nephropathy and neuropathy[4]. This disease remains to be an expanding global health crisis, because it is a potentially morbid condition with high prevalence worldwide thus it constitutes a major health concern[5]. World health organisation (WHO) estimates that by 2030 there will be 333 million diabetics in the world, about 50.8 million in India[6], 11 million in Brazil[7], and 26 million people in U.S population[8]. Due to its high prevalence and potential deleterious effect on a patient's physical and psychological state, diabetes is a major medical concern[9] which, remains incurable and can only be controlled with drugs. Therefore, over the years, several animal models have been developed for evaluating the anti-diabetic activity of various agents. These models include chemical, surgical and genetic manipulations in several animal species[10]. The main drugs used for diabetes mellitus are oral hypoglycemic drugs (Sulphonylureas, Biguanides) but use of these drugs leads to serious side effects like hypoglycaemic shock, lactic acidosis etc[11]. Moreover, the cost of these drugs is also high. Therefore, there is need for better and safer drugs to prevent diabetes in early stages. Herbal products are our national heritage. The uses of herbal remedies are increased many folds from 1990 onwards in USA[12] and other countries. Therefore, the present review is aimed to summarise the different animal models for diabetic mellitus and the plants reported to possess antidiabetic activity.

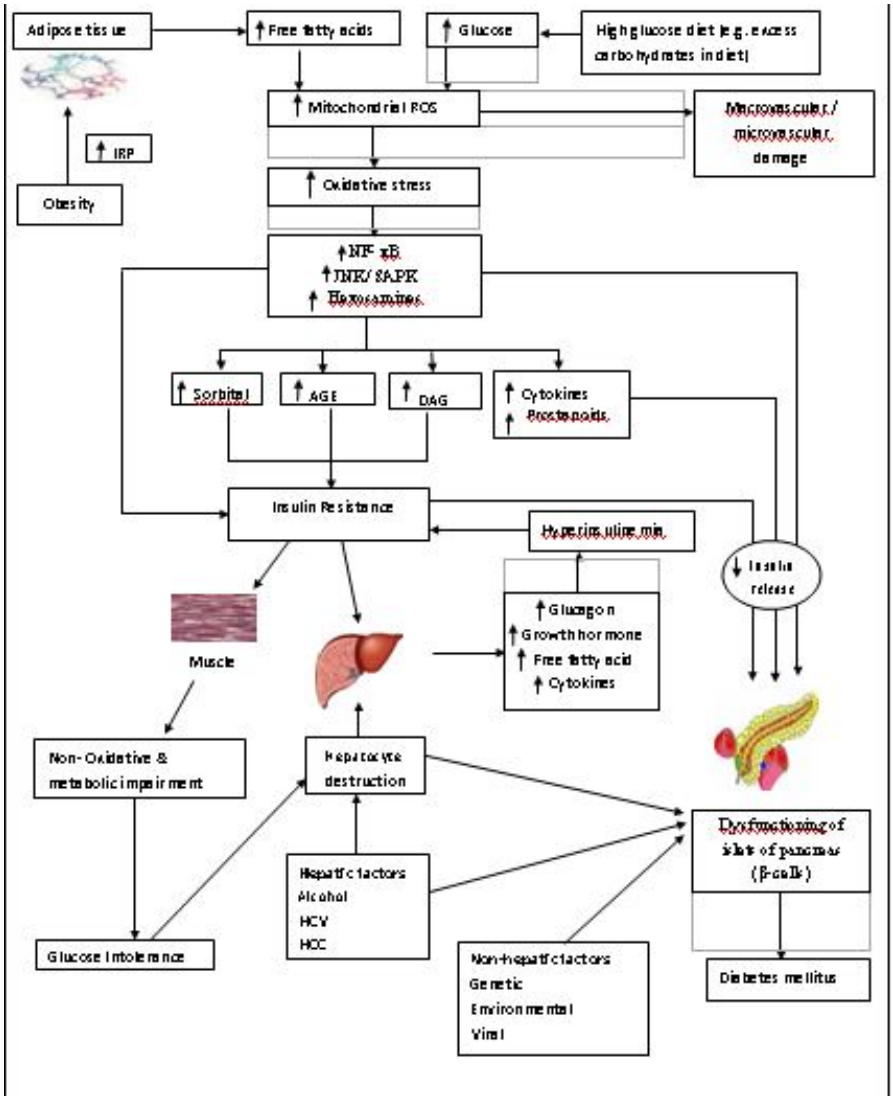
Corresponding Author:
Dr. Anil Kumar Sharma,
Director, CT Institute of Pharmaceutical Sciences,
Shahpur, Jalandhar (Punjab) - 144020, India
Email: aksharma91@gmail.com

1.1 Pathogenesis of Diabetes Mellitus

Insulin is a principle hormone that regulates uptake of glucose from the blood into most of the cells and its deficiency leads to type 2 diabetes mellitus[13]. In type 1 diabetes there is autoimmune destruction of β-cells which is triggered by the factors such as environmental and viral factors[14]. In type 2 diabetes β- cells destruction leads to decrease in insulin release and resistance to insulin develops due to obesity[15]. Adipose tissues are located throughout the body, some of these depots are structural, providing mechanical support and contributing little to energy homeostasis. As the adipose tissue plays an important role in buffering the flux of free fatty acid (FFA) in postprandial period. In obesity there is abnormal production of free fatty acid release due to increased production of inflammation related protein (IRP) in adipose tissue[16]. Thus increased level of FFA and glucose (due to high glucose diet e.g. excessive carbohydrate diet) causes production of mitochondrial reactive oxygen species (ROS). These maladaptive responses, results in oxidative stress and ultimately macro vascular / micro vascular damage[17]. Indirectly mitochondrial ROS produces oxidative stress which functions as a signalling molecule to activate stress sensitive pathways such as nuclear factor ϒB (NF-κB), janus kinases/ stress activated protein kinases (JNK/SAPK), and hexosamines. These stress sensitive pathway increases production of sorbital, advance glycated end product (AGE), cytokines and prostanoids along with diacyl glycerol (DAG) which collectively results in insulin resistance and β-cell dysfunctioning[18]. Insulin resistance results in metabolic impairment of glucose in skeletal muscle (also indirectly involved in hepatocyte damage) and liver. Due to resistance of insulin towards glucose, metabolic impairment takes place which leads to glucose intolerance and hepatocyte destruction. Hepatic factors like alcohol, hepatitis C virus (HCV), hepatocellular carcinoma (HCC) also responsible for hepatic destruction[19] Due to hepatocyte destruction, there is increase in level of glucagon, growth hormone, free fatty acid and cytokines which collectively results in hyperinsulinemia. All above mentioned factors which are indirectly involved in resistance of insulin leads to decreased level of insulin in response to glucose, hence there is dysfunctioning in β-cells of islets in pancreas in finally results in diabetes mellitus[20]. Beyond above mention factors there are

some non-hepatic factors like genetic[21], environmental and viral factors that are directly involved in the diabetes mellitus[22].

Fig.1: Pathogenesis of diabetes.



IRP = Inflammation related protein, ROS = Reactive oxygen species, GAPDH= Glyceraldehyde-3-phosphate dehyrogenase, NF-κB = Nuclear factor κB, JNK/SAPK = Janus kinases/ stress activated protein kinases, IGF-1 = Insulin growth factors, HCV = Hepatitis C virus, HCC= Hepatocellular carcinoma.

2. EXPERIMENTAL ANIMAL MODELS OF DIABETES MELLITUS

Experimental diabetes mellitus is generally induced in laboratory animals by several methods that include: chemical, surgical and genetic (immunological) manipulations. Most of the preliminary experiments in diabetes are carried out in rodents, although some are still performed in larger animals.

The animal models employed for screening anti-diabetic agents can be broadly classified into three types and are enlisted below[23].

1) Methods to induce experimental diabetes mellitus
   - (a) Alloxan
   - (b) Streptozotocin
   - (c) Hormones
   - (d) Viruses induced
   - (e) Other diabetogenic compounds
     - (i) Dithizone
     - (ii) Monosodium glutamate
2) Genetically diabetic animals
   - (a) Spontaneous diabetic rats
   - (b) Spontaneous diabetic mice
   - (c) Other species with inherited diabetic symptoms
3) Miscellaneous models
   - (a) Invertebrate animal model
   - (b) Diet induced metabolic dysregulation

2.1 Methods to Induce Experimental Diabetes Mellitus

2.1.1 Alloxan Induced Diabetes

Alloxan(2,4,5,6-tetraoxypyrimidine; 5, 6-dioxyuracil) is hydrophilic, unstable substance having half life about 1.5 min (at temp 37º C) and is longer at lower temperatures[24] but when a diabetogenic dose is used, the time of alloxan decomposition is sufficient to allow it to reach the pancreas in amounts that are deleterious. Due to selective toxic action of alloxan on β-cells as well as on non β-cells, other non endocrine islets along with extrapancreatic parenchyma, its dose selection should be done precautionally[25].

2.1.2 Streptozotocin Induced Diabetes

STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is cytotoxic especially to β-cells of the pancreas. It is synthesized by *Streptomycetes achromogenes* (species of gram positive

bacteria that belongs to genus Streptomycetes) and is used to induce both type 1 diabetes and type 2 diabetes at dose of 50mg/kg i.p for 3 days[26] and 80-100mg/kg (i.v, i.p or s.c) in neonatal rats for 10days[24]. STZ can also be used in higher animals to induce diabetes given in table-1.

Table - 1: Streptozotocin induced diabetes mellitus in different experimental animal species

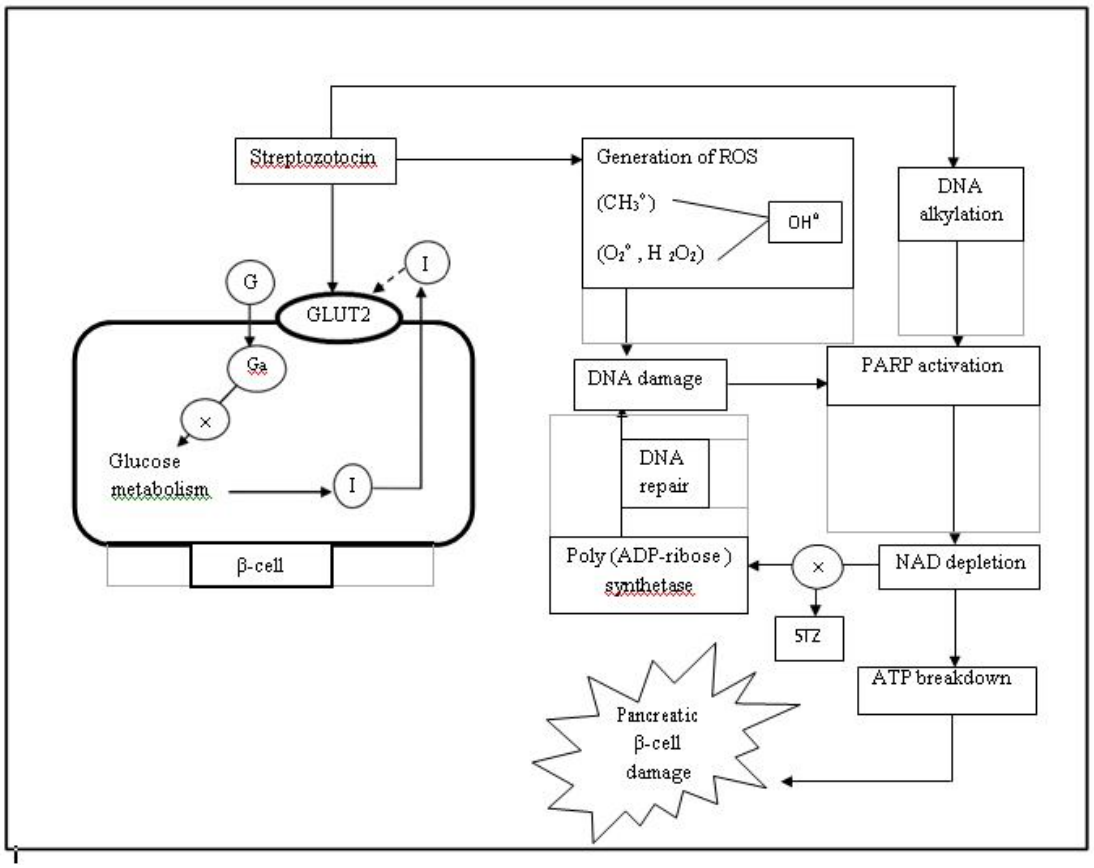
Species	Streptozotocin	Route
Mice	50-200mg/kg	i.v. or i.p[24]
Rat	45-65mg/kg	i.v. or i.p[27]
Hamster	50mg/kg	i.v. or i.p[28]
Dog	20-30mg/kg	i.v. or i.p[29,30]
Pig	100-150mg/kg	i.v. or i.p[31]
Primates	50-150mg/kg	i.v. or i.p[32]

2.1.2.1 Mechanism of Action of Streptozotocin Induced diabetes

It is hypothesized that mechanism of streptozotocin is mediated through the reduction of nicotinamide adenine dinucleotide (NAD) in pancreatic cell. Streptozotocin taken up by pancreatic β-cells via glucose transporter (GLUT2) which prevents the glucose metabolism in cell and results in reduction or inhibition of the insulin release[33, 34]. This leads to generation of reactive oxygen species like methyl radicals (CH_3), oxygen radicals (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) that results in DNA damage[35] which is repaired by an excision process. DNA repair requires activation of NAD dependent enzyme poly (ADP-ribose) synthetase by activation of PARP enzyme. But in STZ, this excision repair process get prevented and results in critical loss of NAD leads to cessation of cellular function by depletion of ATP, eventually β-cell death takes place.DNA alkylation process directly results in NAD depletion which leads to β-cell damage[27]

.

Figure 2: Mechanism of action of Streptozotocin (STZ)



GLUT2= glucose transporter, G= extracellular glucose, G_a= Glucose receptor activated, I = insulin, OH^o= hydroxyl radical, CH_3 = methyl radical, O_2 = oxygen radical, H_2O_2= hydrogen peroxide, DNA= deoxyribonucleic acid, PARP = Poly (ADP-ribose) polymerase, NAD=nicotinamide adenine dinucleotide, ATP=adenosine triphosphate, = inhibition.

2.1.2.2 Histological Studies of Pancreas in STZ Induced Diabetes

In control group there is a normal lobular architecture of pancreas, having abundant islets of langerhans interspersed among the pancreatic exocrine acini, islets appeared lightly stained than the surrounding acinar cells, with intact interlobular connective tissue and interlobular duct (Figure No.3). But in STZ induced diabetic group (Figure No. 4) at 75mg/kg i.p single injection, pancreas shows marked morphological changes after 30 days of induction. The border between endocrine and exocrine region became indistinct. The inflammatory cells surround the ducts, in between the acini and inside the lumen of blood vessel. (Figure No. 5) shows blood vessels contracts and dilates which results in either completely destroyed islets leaving empty space which filled with amyloid like material or nuclear pyknosis and nuclear fragmentation[36].

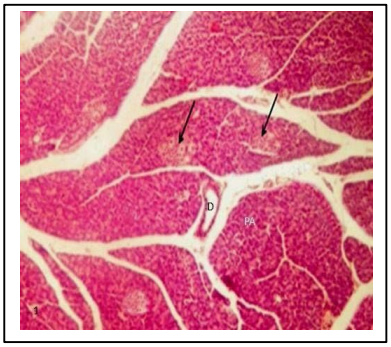


Fig.3: A Photomicrograph of rat pancreatic tissue of the control group showing normal lobular architecture. Islets of Langerhans (black arrows) seen surrounded by the pancreaticacini (PA). Notice the Interlobular connective tissue (CT) and the interlobular duct (D).

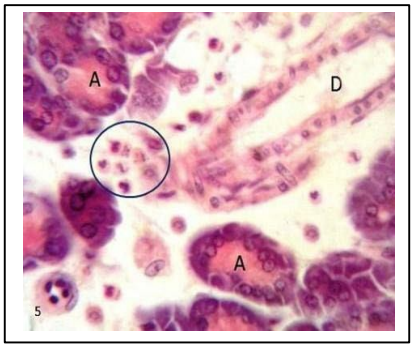


Fig. 4: A photomicrograph of rat pancreatic tissue of the diabetic group showing inflammatory cells (blue circle) surrounding duct (D) and between acini (A).

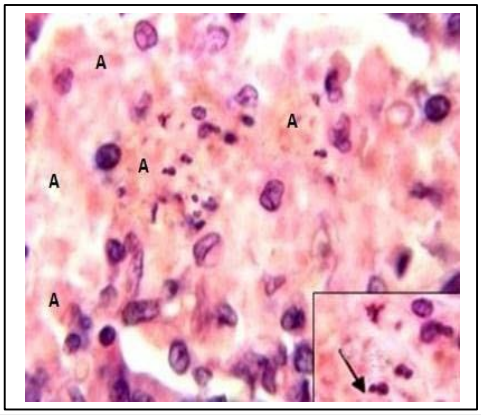


Fig.5: A photomicrograph of rat pancreatic tissue of the diabetic group showing inflammatory cells (blue circle) surrounding duct (D) and between acini (A).

2.1.3 Hormone Induced Diabetes

A number of hormones (e.g. growth hormone, corticosteroids, and thyroxine) modify insulin release in response to glucose. Endocrine pancreas consists of about 1 million microscopic clusters of cells, the islet of langerhans cells, which contains four major cell types i.e β, α, δ and PP (pancreatic polypeptide) cells. The β-cell produces insulin, which is best potent anabolic hormone, with multiple synthetic and growth promoting effects, the α-cell secrete glucagon, inducing hyperglycemia by its glycogenolytic activity in liver[37], the δ-cell secrete somatostatins, which suppress both insulin and glucagon release and PP cells secrete a unique pancreatic polypeptide that exerts several effects like inhibition of gall bladder contraction and pancreatic enzyme secretion.

2.1.3.1 Role of corticosteroids in diabetes mellitus

Steroids possessing both impaired glucose transport in fat and muscle cells, as well as have ability to reduce glucose clearance, which causes a harmful effect on β- cells of the pancreas islet by inducing apoptosis[38].

In dexamethasone, the glucose transporter (GLUT1) protein expression level was decreased, which possibly caused decreased basal glucose uptake and results in insulin resistance. This leads to decreased muscle blood flow, impaired cellular glucose transport, or intracellular deficits of glucose metabolism[38]. At massive dose it produces diabetes only in <20% of wistar rats while in zucker (fa/fa) female rat there is 100% induction of diabetes. Moreover, insulin resistance induced by dexamethasone alone is not sufficient to cause diabetes in wistar rats as in spontaneous NIDDM (non insulin dependent diabetes mellitus) [40].

2.1.3.2 Role of growth hormone in diabetes mellitus

The insulin and growth hormone (GH) or insulin like growth factor -I (IGF-I) axis are two endocrine system that are interlinked at many levels and GH is one of glucose counter regulatory hormone. Therefore, rising in level of GH lead to insulin resistance and hyperglycemia[41]

2.1.3.3 Role of Thyroid hormone (T_3) in diabetes mellitus

Thyroid hormones are widely known for their ability to influence various cellular processes such as mitogenisis and differentiation, which are considered good target for counteracting the diabetes mellitus[42]. Preventive role of T_3 is shown by activation of phosphatidylinositol-3-kinase (PI3k) and Akt or protein kinase B pathway which activate growth factors to stimulate cell growth. This results in β-cell proliferation and survival[43,44].

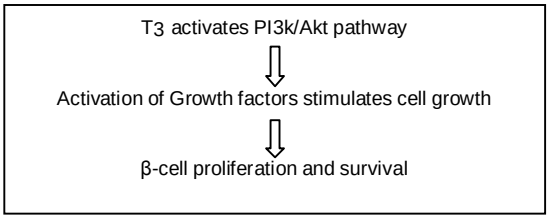


Fig.6: Preventive role of T_3 in Diabetes mellitus

2.1.4 Viruses Induced Diabetes

Viruses are agents that have been thought to be implicated in pathogenesis of Juvenile onset diabetes mellitus and

involved in either direct (infection) destruction of β-cells[45] or by initiation of an autoimmune response to β-cells[23].

Table 2: Different viruses used to induce diabetes in different strains of mouse and their mechanisms of action are given below:

Virus	Strains used (mouse)	Mechanism involved
Coxsackievirus B (CBV)	CBV-3/NANCY CBV-4/J.P.B CBV-5/FAUKNER CVA-9/GRIGGS	Respiratory tract infection → spread throughout lymphatic circulation46
Encephalomyocarditis Virus (EMC)	SJL/L, SWR/J, DAB/1J, DBA/2J, BALB/C	M variant of EMC virus → diabetic like syndrome→ selectively infect pancreatic cells[47,48,49]
Kilham Rat Virus (KRV)	BB-DR	BBDR rats infected with KRV → up regulation of → cytokines → immune response in pancreatic lymph node diabetes [50]
Lymphocytic Choriomeningitis virus (LCMV)	RIP-GP , RIP-NP	Mice infected with LCMV virus → antiviral immune response → mononuclear cell infiltration into islet of pancreas → destruction of β-cells [51]

2.1.5 Insulin Antibodies Induced diabetes

The insulin antibody interaction may be reversible and is dependent on the affinity and capacity of the antibody to bind insulin[52] and several other factors such as genetic factor, insulin purity, insulin aggregation involved in the generation of insulin antibodies[53].

Insulin deficiency mechanism**,** involved as any delay in the initial increase of free insulin level in plasma may causes greater postprandial hyperglycemia because antibody-bound insulin is unavailable to tissues, but the prolongation of postprandial hyperinsulinemia may leads to late hyperglycemia due to antibodies[54].

2.1.6 Other Diabetogenic Compounds

2.1.6.1 Dithizone

Dithizone is sulphur containing organic compound (8-(p-toluene sulfonylamino) quinolone) a good ligand and forms complex with many metals such as lead and mercury[55]. It is used to assess the purity of human pancreatic islet preparation that are used for transplantation in type 1 diabetic subjects[56]. It reacts with zinc in islets of langerhans causing destruction of islet cells and induces diabetes. The mechanism of action of dithizone is based on the formation of an unsaturated (electrophilic) complex with zinc in them[57]. Dithizone induces similar glycemic changes in rabbits, golden hamster and mice (triphasic fluctuations resulting in diabetes)

Initial hyperglycemia observed after 2h
⇩
Normoglycemia after 8h persists for upto 24 h
⇩
Hyperglycemia observed after 24-72 h, last for long period of time
⇩
Diabetes

Fig. 7: Triphasic fluctuations in dithizone induced diabetes

2.1.6.2 Monosodium Glutamate

Monosodium glutamate (MSG) ingestion is known to increase plasma glutamate concentration. MSG infusion stimulates insulin secretion[58]. Subcutaneous administration of MSG to neonatal female non-obese diabetic (NOD) mice resulted in obesity associated with stunting and hyperinsulinemia, this leads to maintaining β-cell function through the modification of degenerative process of islet in the non obese diabetic (NOD) mouse[59].

2.2 Genetically Diabetic Animals

Spontaneous diabetic animals may be obtained from the animals with one or several genetic mutations transmitted from generation to generation (e.g. db/db mice) or by repeated breeding with non-diabetic animals over several generation (BB rat, obese diabetic (TSOD) mouse). These animals generally inherit diabetes either as single or multigene defects[60]. The list of different strains of spontaneous diabetic species and their characteristic features are below in table- 3, 4 and 5.

Table 3: Different strains of spontaneous diabetic rats prone to diabetes and their characteristic features

Strain	Onset of diabetes	Characteristic Features
Biobreeding rat (BB)	7-14 weeks	■ Associated with insulin deficiency and insulitis due to autoimmune destruction of β- cell. ■ Pancreatic islets subjected to an immune attack with T cells, B cells and macrophage recruited to insulitis. ■ Susceptibility of diabetes: Male (M) = Female (F) ■ Auto antibodies: glutamic acid decarboxylase (GAD), islet cell autoantibodies (ICA)[60]
Goto Kakizaki rat (GK)	3- 4 weeks	■ Stable hyperglycemia in adult life span. ■ Both insulin resistance and impaired insulin secretion are present. ■ Associated with type 2 diabetes mellitus[61]
Otsuka Long Evan Tokushima Fatty rat (OLETF)	18 weeks	■ Associated with glucose intolerance[62]. ■ Pancreatic islets undergo 3 stages of histological changes: a) Cellular infiltration (16-20 week old) b) Hyperplasia (20-40 week) c) Finally islet become fibrotic and replaced by connective tissue[63]. ■ Susceptibility of diabetes: M> F[2]
Zucker diabetic fatty rat (ZDF/ DRT-FA)	6-11 weeks	■ Lipotoxicity to β-cells cause diabetes. ■ Used for investigation mechanism associated with insulin resistance and β- cell dysfunction in type 2 diabetes. ■ Frank diabetes due to failure to compensate adequately for insulin resistance. ■ Susceptibility of diabetes : M > F[64, 65]
Zucker fatty rat (ZFR)	4 weeks	■ Zucker (fa/fa) fatty or obese rat results from simple autosomal recessive (fa) gene on chromosome 5. ■ Associated with hyperinsulinemia, hyperlipidemia and hypertensive[65] ■ Impaired glucose tolerance ■ Used for screening different insulin sensitizing and anti obesity agents [64]
James C Russel-LA (JCR: LA)-Corpulent rat	10 weeks	■ Associated with hypertriglyceridemia and hyperinsulinemia with impaired glucose tolerance and also susceptible to vascular arteriosclerotic lesion[66] ■ Used as research model for development of atherosclerotic and myocardial lesions in association with syndrome- X metabolic profile[67]

LEW.1ARI/-iddm 9rats	8-9 weeks	▪ Autoimmune model of diabetes ▪ Exhibits prediabetic period with islet infiltration in a week before animal become hyperglycemic[68]. ▪ Animal survives after the onset of overt diabetes and can be used to study diabetic complications [69]. ▪ Susceptibility of diabetes: M= F[24]
KDP (Komeda diabetes prone) rats	8-16 weeks	▪ In non lymphopenic substrain, frequency of diabetes is 70% and have mild to severe insulitits at 120-220 days of age. ▪ Exhibits lymphocytic infiltration of thyroid and kidney. ▪ In KDP rats, casitas B-lineage lymphoma b (Cblb) function as negative regulator of autoimmunity and a major susceptibility gene for type 1 diabetes in rats[60].
Cohen diabetic rat (CDR)	6 weeks	▪ Genetically derived model of diet induced type 2 diabetes. ▪ Sensitive group developed β- cell dysfunctioning and reduced insulin secretion with insulin resistance[70]
ESS rat	8 weeks	▪ Characterized by abnormal glucose tolerance and consists of mild type of diabetes. ▪ Disruption of the islets architecture and fibrosis of stroma in six month old rats[71].

Table - 4: Different strains of spontaneous diabetic mice prone to diabetes and their characteristic features

Strain	Onset of diabetes	Characteristic Features
Non obese diabetic (NOD) mice	12-30 weeks	▪ Insulitits at age 4-5 week old, followed by subclinical β-cell destruction[72]. ▪ Auto antibodies : GAD, ICA ▪ Susceptibility of diabetes: F> M[73]
Nagoya Shibata Yasuda (NSY) mice	48 weeks	▪ Spontaneous type 2 diabetic model ▪ Impaired insulin secretion. ▪ Susceptibility of diabetes: M> F[74]
Kuo Kondo (KK) mice	8 weeks	▪ Polygenic model of obesity. ▪ Characterized by hyperphagia, hyperinsulinemia, insulin resistance. ▪ Increase in pancreatic insulin content associated with increase in number and size of islet. ▪ Degranulation of β-cells and hypertrophy of islets of pancreas[75].
KK-AY/yellow KK obese mice	5 weeks	▪ Blood glucose and circulating insulin level as well as hemoglobin A1c ▪ (HbA1c) levels are increased from 5 week of age. ▪ Degranulation and glycogen infiltration of β-cell. ▪ Extrapancreatic action of antidiabetic drugs such as glimepiride 23.
AKITA diabetic mice	3-4 weeks	▪ Characterized by hyperglycemia, hypoinsulinemia, polyuria and polydipsia. ▪ Lack of β-cell mass in this model makes it an alternative to STZ treated mice in transplantation studies. ▪ Type 1 diabetes model for macrovascular and neuropathy diabetes2.
Leptin defficint (Lepob/ob) mice	2-4weeks	▪ Model of severe obesity, derives from spontaneous mutation. ▪ Characterized by increase in weight, hyperinsulinemia, hyperglycemia and hyperlipidemia. ▪ Infertile [76,77] ▪ The pancreatic islet volume is dramatically increased in these mice but some abnormalities in insulin secretion although islet maintains insulin secretion and lack of complete β-cell failure shows it not completely type 2 diabetic model 2.
Leptin receptor deficient (Leprdb/db) mice	4-8weeks	▪ Characterized by hyperphagia, obesity, hyperinsulinemia and hyperglycemic. ▪ Significant development of nephropathy 66, 23.

Other species which are used as diabetic model include Sand rat, Spiny mouse, African hamster and TUCO- TUCO (*Ctenomis talarum*).

Table 5: Other species with inherited diabetic symptoms

Species	Characteristic Features
Spiny mouse (*Acomys cahirinus*)	▪ Nocturnal, large light brown mice that weight 30 -50g and having fur brister on their backs. ▪ Diabetes occurs in about 15% of the animals and accompanied by hyperplasia of endocrine pancreas[78]. ▪ Characterized by obesity, mild hyperglycemia and hyperinsulinemia [79].
Sand rat (*Psammomys obesus*)	▪ It is used as model of latent Insulin dependent diabetes mellitus. ▪ Animal developed diabetic symptoms by chow, instead vegetable diet. ▪ Diabetic symptoms develops within 2-3 months and characterized by hyperglycemia and ketosis [80].
African hamaster (*Mystromys albicaudatus*)	▪ Characterized by hyperglycemia, glycosuria, ketonuria, polyuria, polyphagia, polydipsia, pancreatic lesion and β-cell death [81].
TUCO-TUCO (*Ctenomis talarum*)	▪ They are having syndrome similar as in sand rat and spiny mouse. ▪ Characterized by degranulation of β-cell usual lesion in pancreas. ▪ Amyloid hyalinization of islet has been observed. ▪ Less prone to hyperglycemia and ketosis but males become hyperphagic [82].

2.3 Miscellaneous Models

2.3.1 Invertebrate Animal Model

Drosophila model used for understanding metabolism[83] and to study the genetics of metabolic function in various species. Drosophila fly elucidates the pathogenesis of human metabolic disease such as diabetes and obesity. Both diabetic and obese flies, as well as genetically "lean" and hypoglycemic phenotypes have been created as model of human disease[84]. Another invertebrate animal model is Silk Worm (*Bombyx mori*) used for the identification of antidiabetic drugs. The silk worm fed a high glucose diet (10% glucose containing diet) for 3 days shows increases 4 fold in hemolymph sugar level compared with silk worm fed a normal diet[85].

2.3.2 Diet Induced Metabolic Dysregulation

In this model male albino wistar rats are used to induce diabetes with high fructose diet (66 % fructose and 1.1% coconut oil) for 3 weeks shows increase in glucose and glycosylated haemoglobin level86. Another model is non human primates Baboon (*Papio hamadryas*) induced with high sugar, high sugar fat diet after 12 hour fasting. The composition of diet includes 73 % purina monkey chow-5038, 7% lard, 4% crisco, 4% coconut oil, 10.5% flavoured high fructose corn syrup and 1.5 % water. Continuous dietary exposure of 8 weeks increases the body fat and triglyceride levels87.

2.3.2.1 Role of herbs in the treatment of diabetes mellitus

Herbal plants having a potential role in treatment of diabetes as their active principle have been reported to possess pancreatic β-cell regeneration, insulin mimetic activity and also improve insulin resistance. Moreover, due to its cost effectiveness and less side effect these herbal plants are claimed preferred over conventional preparation. The list of different herbal plants reported to possess antidiabetic activity in different models are mentioned in the table 6, 7 and 8.

Table 6: Plants used in STZ induced diabetes model

Sl. No.	Plant name	Common name	Part used/ Type of extract	Observed result
1)	*Agele marmelos* (Rutaceae)	Holy fruit tree	Leaf/Aqueous extract	utilization of glucose[88]
2)	*Aloe vera* (Liliaceae)	Aloe	Whole plant/ Leaf pulp extract	maintain glucose homeostasis [89]
3)	*Annona squamosa* (Annonaceae)	Sugar apple	Leaf/ ethanolic leaf extract	blood glucose level [90]
4)	*Andrographis paniculata* (Acanthaceae)	King of bitter	Whole plant/ aqueous extract	prevent glucose absorption [91]
5)	*Azadirachta indica* (Meliaceae)	Neem	Leaf/aqueous extract	hypoglycemic activity [92]
6)	*Cassia auriculata* (leguminosae)	Tanner's cassia	Flower/ aqueous extract	enhanced utilization of glucose [93]
7)	*Caesalpinia bonducella* (Caesalpinoidiae)	Chinese cinnamon	Seed/aqueous and ethanolic extract	release of insulin [94]
8)	*Citrullus colocynthis* (Cucurbitaceae)	Bitter apple	Seed/ aqueous extract	level of Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)and shows hypoglycemic activity [92]
9)	*Casearia esculenta* (Flacourtiaceae)	Carilla	Root/aqueous extract	blood glucose level [95]
10)	*Catharanthus roseus* (Apocynaceae)	Madagascar periwinkle	Leaf and twigs/ ethanolic leaf extract	mobilization of glucose [96]
11)	*Eugenia jambolana* (Myrtaceae)	Indian black berry	Seed powder/ ethanolic seed extract	shows better glucose tolerance 6
12)	*Morus alba* (Moraceae)	White mulberry	Leaf/ aqueous extract	glucose uptake [97]
13)	*Mangifera indica* (Anacardiaceae)	Mango	Leaf/ aqueous extract	glucose absorption [98]
14)	*Ocimum sanctum* (Lamiaceae)	Holy Basil	Whole plant, leaf/ leaf powder extract	glucose level [99]
15)	*Punica granatum* (Punicaceae)	Pomegranate	Flower/ methanolic seed and flower exract	blood glucose level[100]

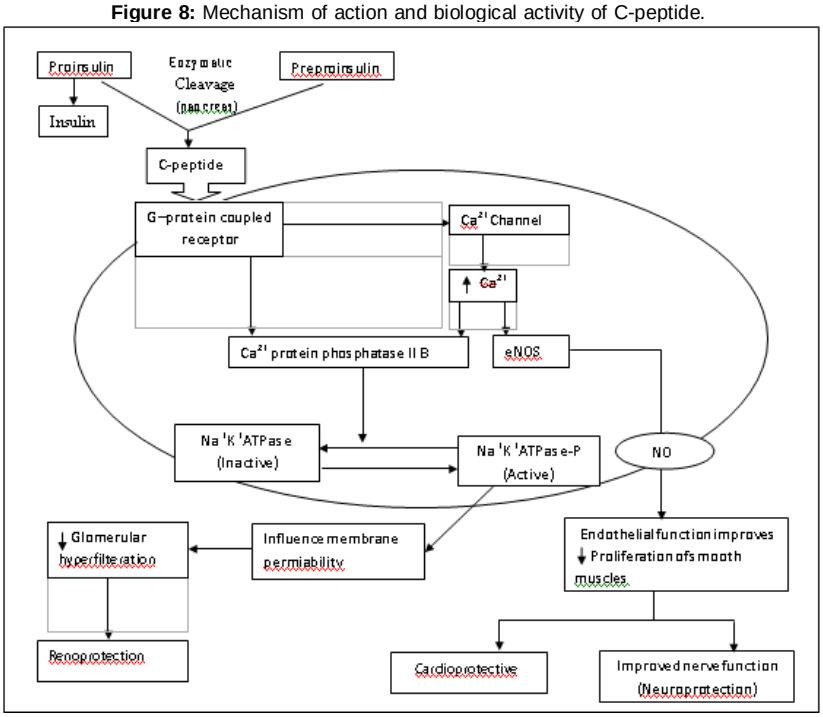
Table 7: Plants used in High fructose diet induced diabetes model

S no.	Plant name	Common name	Part used/Type of extract	Observed result
1)	*Anacardium occidentale* (Anacardiaceae)	Cashew plant	Stems and Bark/ methanolic extract	blood glucose level[101]
2)	*Amaranthus cruentus* (Amaranthaceae)	Amarath	Seeds/aqueous extract	malondialdehyde level in plasma [102]
3)	*Andrographis paniculata* (Acanthaceae)	Chieretta	Whole plant, Leaf and Stem/ethanolic extract	antidiabetic andantioxidant activity[103]
4)	*Allium sativum* (Liliaceae)	Garlic	Bulbs/ Homogenates	improve insulin sensitivity[104]
5)	*Azadirachta indica* (Maliaceae)	Neem	Leaves/ aqueous extract	improve glucose intolerance[105]
6)	*Fagopyrum tataricum* (Polygonaceae)	Buck wheat	Roots /powder ethanolic extract	glucose and triglyceride level[106]
7)	*Ficus exasperata* (Moraceae)	Vahl	Leaves aqueous extraction	ameliorated glucose tolerance[107]
8)	*Gymnema sylvestre* (Asclepiadaceae)	Gurmar	Leaves/ aqueous extraction	glucose and cholestrol level[108]
9)	*Ibervillea sonorae* (Cucurbitaceae)	Wareke	Roots/powder extract	glucose level[109]
10)	*Salacia chinensis* (Celastaceae)	Modhupal	Leaves/aqueous extract	serum glucose level[108]
11)	*Syzgium cumini* (Myrtaceae)	Jamun	Roots/powder extract	serum glucose level[108]
12)	*Tamarindus indica* (Caesalpiniaceae)	Tamarind	Seeds/aqueous extract	Low density lipoprotein (LDL) and high density lipoprotein (HDL) level[110]
13)	*Trigonella foenum graecum* (Leguminosae)	Fenugreek	Seeds/aqueous extract	improved insulin sensitivity[111]
14)	*Vitis vinifera* (Vitaceae)	Grapes	Seeds/ aqueous extract	prevent insulin resistance[112]

Table 8: Supportive plants used in diabetes, as artificial sweeteners

S. no.	Plant name	Common name	Part used/ Type of extract	Observed result
1)	*Glycyrrhiza glabra* (Fabaceae)	Liquorice	Roots/ ethanolic extract	LDL level and HDL level[113]
2)	*Stevia rebaudiana* (Compositae)	Sweet leaf of paraguay	Leaves/aqueous extract	antihyperglycemic, antihypertensive and anti human rotavirus activity[114]

Figure 8: Mechanism of action and biological activity of C-peptide.



Ca^{2+} = calcium ion, eNOS = endothelial nitric oxide synthase, NO= Nitrous oxide, Na^{+} K^{+} ATPase- P = sodium potassium ATPase channel phosphorylation.

3. BIOMARKERS OF DIABETES

Biomarker is defined as "biological molecule that represents health and disease state" measured in body fluids. In blood HbA1c, blood glucose level, triglyceride levels, cholestrol level, blood urea level, and creatinine and ketone levels are measured to study the diabetic state. In addition to this, now a days C- peptide is also considered as important biomarker for determining the level of diabetes[115].

3.1 C-Peptide

C-peptide is a polypeptide with molecular weight (MW) 3600, containing 31amino acid. Degradation of the C-peptide is mainly takes place in kidney and half life of C-peptide in circulation is 2-5 time longer than insulin. It is generally known that C-peptide precursors having little or no biological activity but currently available information establishes that C- peptide is not biologically inert, but having its own physiological effects[116].

3.1.1 Mechanism of action and biological activity of C-Peptide

Peptide is produced by a series of enzymatic cleavage of the precursor molecule preproinsulin and pro insulin, in pancreas[116]. C-peptide acts through G-protein coupled receptor to activate calcium-dependent signalling pathway[117]. This calcium signalling pathway is thought to activate the inactive form of Na+ K+ ATPase enzyme through phosphorylation of Phosphatase II B Ca+ protein, ultimately influence the membrane permeability and leads to decreased glomerular hyperfilteration and results in renoprotection. Moreover, increase in intracellular calcium level through calcium channel leads to activation of endothelial nitric oxide synthase (eNOS) which result in release of nitrous oxide (NO) from endothelial membrane and this NO decreased vascular and smooth muscle proliferation and improve the endothelial function. This improved endothelial function results in the reduction of neural and cardiac dysfunctioning, which reveals a protective action of C-peptide in neural and cardiac dysfunctioning[118, 116.]

3.1.2 Physiological role of C-peptide

Peptide having the capacity to diminish glomerular hyperfilteration and reduce urinary albumin excretion[119] , enhanced Na+, K+-ATPase activity in neuropathy[120] and prevents vascular dysfunctioning118 in experimental and type 1 diabetes[119].

3.2 Glycated Hemoglobin

Glycated hemoglobin (GHb) is another biomarker used in diabetes mellitus. It is formed by the non- enzymatic addition of a sugar residue and it consists of Hemoglobin A1 (90-95%), A2 (2-3%), F (0.5%), A1a (1.6%), A1b (0.8%) and A1c (3-6%) [121]. The conversion of HbA to HbA1c takes place during entire life span of red blood cells, so concentration of HbA1c is higher in old red cells[120]. GHb in blood is the only protein that widely used to monitor glycemic control [122]. According to Americans Diabetic association, in normal adults Hb1Ac should be between 5-6 %. In diabetics, higher amount of glycated Hb is observed, indicating poorer control of blood glucose level, and associated with complications of cardiovascular disease, nephropathy, neuropathy and retinopathy[123].

REFERENCES

1. Ogbonnia Steve O, Adekunle A, et al "Assessing plasma glucose and lipid levels, body weight and acute toxicity following oral administration of an aqueous ethanolic extract of *Parinari curatellifolia* Planch, (Chrysobalanaceae) seeds in alloxan-induced diabetes in rats ", *African Journal of Biotechnology, 2008*, 7 (17): 2998-3003.

2. King JF Aileen, "The use of animal models in diabetic research" *British Journal of Pharmacology*, *2012*, 166: 877-894.
3. Fowler Michael J, "Microvascular and macrovascular complications of diabetes" *Clinical diabetes*, *2008*, 26 (2): 77-82.
4. Mohd. Muneer Ahamed and Otilia Banji, "A review on diabetic neuropathy and nephropathy" *International journal of pharmaceutical sciences and research*, *2012*, 3 (2): 300-304.
5. Hjelm K, Mufunda E, et al "Preparing nurse to face the pandemic of diabetes mellitus: a literature review" *J.Ada.Nurs, 2003*, 41 :424-434.
6. Ramachandran A, Das A K, "Current status of diabetes in India and need for novel therapeutic agents" *JAPI, 2010* :58, 7-9.
7. Andrade Regina celia Gracia de, Figueiredo Roberta Carvalho de, et al "Prevelance of diabetes mellitus in the Japanese–Brazilian community of Mombuca, Guatapara , SP" *Arq Bras Endocrinol Metab, 2011*, 55 (2): 127-133.
8. National Diabetes Statistics, National institute of health, 2011, 3892 (11) :1-12.
9. Macedo CS, Capelleti SM, et al "Role of metabolic control on diabetic nephropathy" *Acta Cir Bras, 2002*, 17(6): 37-5.
10. Etuk EU, "Animal models for studying diabetes mellitus" *Agric Biol J N Am,2010*, 1 (2):130-134.
11. Bastaki Salim, "Review Diabetes mellitus and its treatment" *Int J Diabetes and Metabolism, 2005,* 13 : 111-134.
12. Mohammadi Jamshid, Naik Prakash R, "Evaluation of hypoglycemic effect of Morus alba in an animal model" *Indian J Pharmacol, 2008*, 40 (1): 15-18.
13. Ebesunun Maria O, Adetunji Kehinde, et al "Evaluation of essential fatty acids, folic acid and vitamin B12 in type 2 diabetes mellitus" *New York Science Journal, 2012*, 5 (8): 56-64.
14. Joshi SK, Shrestha S, "Diabetes mellitus: A review of its associations with different environmental factors" *Kathmandu University Medical Journal, 2010*, 8 (29): 109-115.
15. Larsen CM, Faulenbach M, et al "Interleukin-1-receptor antagonist in type 2 diabetes mellitus" N *Engl J Med*, 2007, 356 (15): 1517–1526.
16. Tataranni PA, "Pathophysiology of obesity- induced insulin resistance and type 2 diabetes mellitus" *European review for medical and pharmacological sciences, 2002*, 6: 27-32.
17. Naudi Alba, Jove Mariona, et al "Cellular dysfunction in diabetes as maladaptive response to mitochondrial oxidative stress" *Experimental Diabetes Research, 2012*: 1-14.
18. Mohamed AK, Bierhaus A, et al "The role of oxidative stress and NF-κB activation in late diabetic complications" *Biofactors, 1999*, 10: 157–167.
19. Levinthal Gavin N, Tavill Anthony S, "Liver Disease and Diabetes Mellitus" *Clinical Diabetes, 1999*, 17 (2): 85.
20. Wiesenthal Stephanie R, Sandhu Harmanjit, et al "Free fatty acids impair hepatic insulin extraction in vivo" *Diabetes, 1999*, 48: 766-774.
21. Guja Cristian, Gagniuc Paul, et al "Genetic factors involved in the pathogenisis of type 2 diabetes"*Proc Rom Acad, 2012*: 44-61.
22. Knip Mikael, Veijola Ritta, et al "Enviornmental triggers and determinants of type 1 diabetes" *Diabetes, 2005*, 54 (2): S125- 136.
23. Kumar Suresh, Singh Rajeshwar, et al "Acute and chronic animal models for the evaluation of anti- diabetic agents" *Cardiovascular Diabetology, 2012*, 11 (9): 1-13.
24. Bonner-Weir S, Trent DF, et al "Responses of neonatal rat islets to streptozotocin; Limited B cell regeneration and hyperglycemia" *Diabetes, 1981*, 30 (1): 64-69.
25. Rohilla Ankur, Ali Shahjad, "Alloxan Induced Diabetes: Mechanisms and Effects" *International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012*, 3 (2): 819-823.
26. Mondal Suvankar, Bhattacharya Sanjib, et al "Antidiabetic activity of *Areca catechu* leaf extracts against streptozotocin induced diabetic rats" *Journal of Advanced Pharmacy Education and Research, 2012*, 2 (1): 10-17.
27. Sharma Vivek Kumar, "Streptozotocin: A experimenat tool in diabetes and Alzimer's disease" *International Journal of Pharma Research and Development, 2009*, 2 (1): 1-7.
28. Rerup CC, "Dugs producing diabetes through damage of the insulin secreting cells" *Pharmacol Rev, 1970*, 22 (4): 485-518.
29. Miller DL, "Experimental diabetes: Effect of streptozotocin on golden Syrian hamster" *Lab Anim Sci, 1990*, 40 (5): 539-540.
30. Battell ML, Yuen VG, et al "Other models of type 1 diabetes In: McNeil JH (Ed). Experimental models of diabetes" *Florida, 1999*: 219-229.
31. Grussner R, Nakhleh R, et al, "Streptozotocin-induced diabetes mellitus in pigs" *Horm Metab Res, 1993,* 25 (4): 199-203.
32. Theriault BR, Thistlethwaite JR, et al "Induction, maintenance, and reversal of streptozotocin- induced insulin- dependent diabetes mellitus in the juvenile cynomolgus monkey (*Macaca fascilularis*)" *Transplantation, 1999*, 68 (3): 331-337.
33. Schnedl WJ, Ferber S, et al "STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells" *Diabetes, 1994*, 43 (11): 1326-1333.
34. Thulesen J, Orskov C, et al "Short-term insulin treatment prevents the diabetogenic action of streptozotocin in rats" *Endocrinology, 1997*, 138 (1): 62-68.
35. Bolzan Alejandro D, Bianchi Martha S, "Genotoxicity of Streptozotocin" *Mutation Research,2002*, 512 (2002): 121–134.
36. Rifaai Rehab Ahmed, El-Tahawy Nashwa Fathy, et al "Effect of quercetin on the endocrine Pancreas of the experimentally induced diabetes in male albino rats: A histological and immunohistochemical Study" *Open Access Scientific Reports*, *2012*, 1 (6): 1-11.
37. Kumar Vijay , Abbas Abul, Pathologic basic of disease, 7th Edition, Saunders, Philadelphia, 2006, 1189.
38. Lansang M Cecilia, Hustak Leighanne Kramer, "Glucocorticoids-induced diabetes and adrenal suppression: How to detect and manage them" *Cleveland Clinic Journal of medicine, 2011*, 78 (11): 748-756.
39. Tappy L, Randin D, et al "Mechanism of Dexamethasone induced insulin resistance in healthy human" *J clin endocrinol metab, 1994*, 79 (4): 1063-1069.
40. Ogawa Atsushi, Johnson John H, et al "Roles of Insulin Resistance and β-Cell dysfunction in Dexamethasone-induced Diabetes" *The Journal of Clinical Investigation, 1992*, 90: 497-504.
41. Holly JM, Amiel SA, et al "The role role of growth hormone in diabetes mellitus" *J Endocrinol, 1988*, 118 (3): 353-364.
42. Falzacappa Cecilia Verga, Mangialardo Claudia, et al "Thyroid hormone T3 counteracts STZ induced diabetes in mouse" *PLoS ONE, 2011*, 6 (5): e19839.
43. Elghazi L, Rachdi L, et al "Regulation of β-cell mass and function by the Akt/protein kinase B signalling pathway" *Diabetes Obes Metab, 2007*, 9 (2): 147–157.
44. Leibiger IB, Leibiger B, et al "Insulin signaling in the pancreatic β-cell" *Annu Rev Nutr, 2008*, 28: 233–251.
45. Jun HS, Yoon JW, "A new look at viruses in type 1 diabetes" *Diabetes Metab Res Rev, 2003*, 19 (1) : 8–31.
46. Merja Roivainem , Suvi Rasilainen, et al "Mechanism of Coxsackievirus-induced damage to human pancreatic β-cells" *The Journal of clinical Endocrinology and Metabolism, 2000*, 85 (1): 432-440.
47. Bae Y S and J W Yoon "Determination of diabetogenicity attributable to a single amino acid, Ala-

776, on the polyprotein of encephalomyocarditis virus" *Diabetes, 1993*, 42 (3): 435–443.
48. Smirnov YA, Rodrigues-Molto MP, et al "Protein-RNA Interaction in Encephalomyocarditis Virus as revealed by UV light-induced covalent linkages" *J Virol, 1983*,45 (3): 1048-1055.
49. Craighead JE, Stenke J "Diabetes mellitus like syndrome in mice infected with encephalomyocarditis virus" *Am J Pathol, 1971*, 63 (1): 119-134.
50. Zipris DE. Lien Xie JX, et al "TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats" *J Immunol, 2005*, 174 (1): 131–142.
51. Oldstone MB, Nerenberg M, et al "Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response" *Cell, 1991*, 65 (2): 319-331.
52. Kim MR, Sheeler LR, et al "Insulin antibodies and hypoglycemia in diabetic patients. Can a quantitative analysis of antibody binding predict the risk of hypoglycemia?" *Endocrine, 1997*, 6 (3): 285-291.
53. Jin Ook Chung, Dong Hyeok Cho, et al "Spontaneous hypoglycemia due to Insulin antibody after Insulin treatment of Diabetic Ketoacidosis" *Endocrinol Metab, 2010*, 25 (3): 217-220.
54. Van Haeften TW, Bolli GB, et al "Effect of insulin antibodies and their kinetic characteristics on plasma free insulin dynamics in patients with diabetes mellitus" *Metabolism, 1986*, 35 (7): 649-656.
55. Billman John H, Cleland Elizabeth S, "Dithizone" Org Synth Coll, 1955, 3: 360.
56. Ricordi C, Gray DW, et al "Islet isolation assessment in man and large animals" *Acta diabetologica latina, 1990*, 27 (3): 185–195.
57. Toroptsev IV, Eshchenko VA, et al , Biol Med, 1982, (7): 100.
58. Graham TE, Sgro V, et al "Glutamate ingestion: the plasma and muscle free amino acid pools of resting humans" *Am J Physiol Endocrinol Metab, 2000*, 278 (1): E83-89.
59. Nakajima H, Tochino Y, et al "Decreased incidence of diabetes mellitus by monosodium glutamate in the non-obese diabetic (NOD) mouse." *Res Commun Chem Pathol Pharmacol, 1985*, 50 (2): 251- 257.
60. Mordes John P, Bortell Rita, et al "Rat Models of Type 1 Diabetes: Genetics, Environment, and Autoimmunity" *ILAR Journal, 2004*, 45 (3): 278-921.
61. Mrialles F, Portha B, "Early development of beta cells is impaired in the GK rat model of type 2 diabetes" *Diabetes, 2001*, 50 (1): S84–88.
62. Kawano K, Hirashima T, et al "Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima fatty (OLETF) strain" *Diabetes, 1992*, 41 (11): 1422– 1428.
63. Kose H, Moralejo DH, et al "Examination of OLETF-derived non-insulin-dependent diabetes mellitus QTL by construction of a series of congenic rats" *Mamm Genome, 2002*, 13 (10): 558–562.
64. Himms-Hagen J, Danforth E Jr, "The potential role of β3 adrenergic agonists in the treatment of obesity and diabetes" *Curr Opin Endocrinol, Diabetes, 1996*, 3: 59-65.
65. Srinivasan K, Ramarao P, "Animal models in type 2 diabetes research: An overview" *Indian J Med Res, 2007*, 125 (3): 451-472.
66. Raina Gurudev Singh, "Obesity being the major health burden needed to be chased: A systemic review" *Journal of Applied Pharmaceutical Science, 2011*, 1 (6): 238-245.
67. Clark TA, Pierce GN, "Cardiovascular complications of non-insulin-dependent diabetes; the JCR:LA-cp rat" *J Pharmacol Toxicol Methods, 2000*,43 (1): 1-10.
68. Jorns A, Gunther A, et al "Immune cell infiltration, cytokine expression, and beta-cell apoptosis during the development of type 1 diabetes in the spontaneously diabetic LEW.1AR1/Ztm-iddm rat" *Diabetes , 2005,* 54 (7): 2041–2052.
69. Mathews CE, "Utility of murine models for the study of spontaneous autoimmune type 1 diabetes" *Pediatr Diabetes, 2005*, 6 (3): 165–177.
70. Weksler-Zangen S, Yagil C, "The newly inbred Cohen diabetic rat. A nonobese normolipidemic genetic model of diet induced type 2 diabetes expressing sex differences" *Diabetes, 2001*: 50 (11), 2521-2529.
71. Dumm Clag, Semino MC, et al "Sequential changes in pancreatic islets of spontaneously diabetic rats" *Pancreas, 1990*, 5 (5): 533-539.
72. Yoon JW, Yoon CS, et al "Control of autoimmune diabetes in NOD mice by GAD expressison or suppression in β cells" *Science, 1999*, 284 (5417): 1183–1187.
73. Hammond KJ, Poulton LD, et al "Alpha/beta-T cell receptor (TCR)+CD4-CD8- (NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10" *J Exp Med, 1998*, 187 (7): 1047-1056.
74. Ueda H, Ikegami H, et al "The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity" *Diabetologia, 1995*, 38 (5): 503–508.
75. Reddi AS, Camerini-Davalos RA, "Hereditary diabetes in the KK mouse: an overview" *Adv Exp Med Biol, 1988*, 246: 7-15.
76. Lindstrom P, "The physiology of obese-hyperglycemic mice [ob/ob mice]" *Scientific world journal, 2007*, 7: 666–685.
77. Chehab FF, Lim ME, et al "Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin" *Nat Genet,1996*, 12: 318–20.
78. Pictet R, Orci L, et al "Ultrastructural studies of the hyperplastic islets of Langerhans of spiny mice (*Acomys cahirinus*) before and during the development of hyperglycemia" *Diabetologia, 1967*, 3: 188-211.
79. Hackel DB, Schmidt-Nielsen K, et al "Diabetes mellitus in the sand rat (*Psammomys obesus*): Pathological studies" *Lab Invest, 1965*, 14: 200-207.
80. Dubault J, Boulanger M, et al "Latent autoimmune diabetes mellitus in adult humans with non- insulin-dependent diabetes: is Psammomys obesus a suitable animal model?" *Acta Diabetol, 1995*, 32 (3): 92-94.
81. Jones E, "Spontaneous hyperplasia of the pancreatic islets associated with glycosuria in hybrid mice. In the structure and metabolism of pancreatic islets" *Oxford, 1964*: 189-191.
82. Schmidt G, Martin AP, et al "Evaluation of hepatic mitochondrial function in the spontaneously diabetic *Mystromys albicaudatus*" *Lab Invest , 1974*, 30 (4): 451-457.
83. Baker KD, Thummel CS "Diabetic larvae and obese flies Emerging studies of metabolism in Drosophila" *Cell Metab, 2007*, 6 (4): 257-266.
84. Bharucha KN, "The epicurean fly : Using Drosophila melanogaster to study metabolism" *Pediatric Research, 2009*, 65 (2): 132-137.
85. Matsumoto Yasuhiko, Sumiya Eriko, et al "An invertebrate hyperglycemic model for the identification of anti-diabetic drugs" *PLoS ONE, 2011*, 6 (3), 1-12.
86. Jayanthi M, Jegatheesan K, et al "Hypoglycemic effect of 2-hydroxychalcone on high fructose fed diabetic rat" *International journal of pharmaceutical sciences and research*, *2012*, 3 (2): 600-604.
87. Higgins PB, Bastarrachea RA, et al "Eight week exposure to a high sugar high fat diet results in adiposity gain and alterations in metabolic biomarkers in baboons (*Papio hamadryas sp*)" *Cardiovascular Diabetology, 2010*, 9:71.
88. Sachdewa A, Raina D, et al "Effect of *Aegle marmelos* and *Hibiscus rosa sinensis* leaf extract on glucose tolerance in glucose induced hyperglycemic rats (Charles foster)" *J Environ Biol, 2001*, 22 (1): 53–57.
89. Rajasekaran S, Sivagnanam K, et al "Hypoglycemic effect of Aloe vera gel on streptozotocin- induced diabetes in experimental rats" *J Med Food, 2004*, 7 (1): 61–66.
90. Gupta RK, Kesari AN, et al "Hypoglycemic effect of ethanolic extract of leaves of *Annona squamosa L*. in

experimental animals" *J Ethnopharmacol, 2005*, 99 (1): 75–81.
91. Hung Yu BC, Chen CR WC, et al "Antihyperglycemic effect of andrographolide in streptozotocin- induced diabetic rats" *Planta Medica, 2003*, 69 (12): 1075–1079.
92. Singh Ayodhya, Singh Kusum, et al "Hypoglycaemic activity of different extracts of various herbal plants" *International Journal of Research in Ayurveda and Pharmacy, 2010*, 1 (1): 212-224 .
93. Abesundara KJ, Matsui T, et al "Alpha-glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, Cassia auriculata, exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose" *Journal of Agricultural and Food Chemistry, 2004*, 52: 2541–2545.
94. Chakrabarti S, Biswas TK, et al "Hypoglycemic activity of *Caesalpinia bonducella F* in chronic type 2 diabetic model in Long-Evans rats and evaluation of insulin secretagogue property of its fractions on isolated islets" *J Ethnopharmacol, 2005*, 97 (1): 117–122.
95. Prakasam, A, Sethupathy S, et al "Antihyperglycaemic effect of *Casearia esculenta* root extracts in streptozotocin-induced diabetic rats" *Pharmazie, 2002*, 57 (11): 758–760.
96. Singh SN, Vats P, et al "Effect of an hypoglycemic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats" *J Ethnopharmacol, 2001*, 76 (3): 269–277.
97. Chen F, Nakashima N, "Hypoglycemic activity and mechanisms of extracts from mulberry leaves (*Folium mori*) and cortex mori radicis in streptozotocin-induced diabetic mice" *Yakugaku Zasshi, 1995*, 115 (6): 476–482.
98. Murugananda S, Srinivasan K, et al "Effect of *Mangifera indica* on hyperglycemia and atherogenicity in streptozotocin diabetic rats" *J Ethnopharmacol, 2005*, 97 (3): 497–501.
99. Vats V, Yadav SP, et al "Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats" *J Ethnopharmacol, 2004*, 90 (1): 155–160 .
100. Das AK, Mandal SC, et al "Studies on the hypoglycaemic activity of *Punica granatum* seed in streptozotocin induced diabetic rats" *Phytotherapy Research, 2001*, 15 (7): 628–629.
101. Olatunji Lawrence A, Okwusidi John I, et al "Antidiabetic effect of *Anacardium occidentale* Stem- Bark in Fructose-Diabetic Rats" *Pharmaceutical Biology, 2005*, 43 (7): 589–593.
102. Pasko P, Barton H, et al "Effect of amaranth seed (*Amaranthus Cruentus*) in the diet on some biochemical parameters and essential trace elements in blood of high fructose- fed rats" *Nat Prod Res, 2011*,25 (8): 844-849.
103. Nugroho Agung Endro, Andrie Mohamad, et al "Antidiabetic and antihiperlipidemic effect of *Andrographis paniculata* (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats" *Indian J Pharmacol, 2012*, 44 (3): 377-381.
104. Padiya Raju, Khatua Tarak N, et al "Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats" *Nutrition and Metabolism, 2011,*8 (53): 1-8.
105. Srivastava Shikha, Lal Vijay Kumar, et al "Polyherbal formulations based on Indian medicinal plants as antidiabetic phytotherapeutics" *Phytopharmacology, 2012*, 2 (1): 1-15.
106. Lee Chia-Chen, Hsu Wei-Hsuan, et al "*Fagopyru mtataricum* (Buckwheat) Improved High- Glucose-Induced Insulin Resistance in Mouse hepatocytes and diabetes in Fructose-Rich Diet- Induced Mice" *Experimental Diabetes Research, 2012*: 1-10.
107. Taiwo Idowu Adewumi, Adebesin Olumide Adedapo, et al "Glycaemic Activity of *Ficus exasperata* in Fructose-Induced Glucose Intolerance in Rats" *Researcher, 2010*, 2 (1): 80-83.
108. Neeharika V, Vamsi Krishna R, et al "Effect of Madhuriktha on Dexamathasone and Fructose Induced Insulin Resistance in Rats" *J Nat Prod Plant Resour, 2012*, 2 (2): 288-294.
109. Rivera-Ramirez Fabiola, Escalona-Cardoso Gerardo N, et al "Antiobesity and hypoglycaemic effects of aqueous extract of *Ibervillea sonorae* in mice fed a high-fat diet with fructose" *Journal of Biomedicine and Biotechnology, 2011*: 1-6.
110. Shahraki Mohammd Reza, Harati Mehdi, et al "Prevention of high fructose-induced metabolic syndrome in male wistar rats by aqueous extract of *Tamarindus indica* Seed" *Acta Medica Iranica, 2011*, 49 (5): 277-283.
111. Kannappan S, Anuradha CV, "Insulin sensitizing actions of fenugreek sseed polyphenols, quercetin and metformin in a rat model" *Indian J Med Res, 2009*, 129 (4): 401-408.
112. Suwannapheta Wannaporn, Meepromb Aramsri, et al "Preventive effect of grape seed extract against high-fructose diet-induced insulin resistances and oxidative stress in rats" *Food and Chemical Toxicology, 2010*, 48 (7): 1853–1857.
113. Kanwal Raj , Maurya Santosh Kumar, et al "Antidyslipidaemic activity of *Glycyrrhiza glabra* in high fructose diet induced dsyslipidaemic syrian golden hamsters" *Indian Journal Of Clinical Biochemistry, 2009*, 24 (4): 404-409.
114. Junbi Hend HA, Amer Afaf HB, "Biological properties of Stevia sweetener and egg replacers' products on serum biochemical markers of diabetic rats" *Int J Nutr Metab, 2010*, 2 (5): 82-87.
115. Lyons TJ, Basu A, "Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers" *Transl Res, 2012*, 159 (4): 303–312.
116. Marques RG, Fontaine MJ, et al "C-peptide: much more than a byproduct of insulin biosynthesis" *Pancreas, 2004,* 29 (3): 231-238.
117. Wahren J, Ekberg K, et al "Role of C-peptide in human physiology" *Am J Physiol, 2000*, 278: E759–768.
118. Lina Nordquist, Fredrik Palm, et al "Renal and vascular benefits of C- peptide: molecular mechanism of C-peptide action" *Biologics: Targets and Thearphy, 2008*, 2 (3): 441-452.
119. Chowta M N, Adhikari PH, et al "Serum C-peptide level and renal function in diabetes mellitus" *Indian J nephrol, 2010*, 20 (1): 25-28.
120. Cotter Mary A, Ekberg Karin, et al "Effect of proinsulin C-peptide in experimental diabetic nephropathy" *Diabetes, 2003*, 52: 1812-1817.
121. Nanji Amin A, Pudek Monis R, "Glycosylated Hemoglobins: A Review" *Can Fam Physician, 1983*, 29: 564-568.
122. Gilbert RE, Goodall I, et al "Interlaboratory variation of GHb assays in Victoria, Australia" *Diabetes Care, 1996*, 19: 730-734 .
123. Mahajan Rajni Dawar, Mishra Bhawesh, "Using glycated hemoglobin HbA1c for diagnosis of diabetes mellitus: An indian perspective" *Int J Biol Med Res, 2011*, 2 (2): 508-512.