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Review Article

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

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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 become 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 hyperglycemia¹. 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 disease², retinopathy³, nephropathy and neuropathy⁴. 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 concern⁵. World health organisation (WHO) estimates that by 2030 there will be 333 million diabetics in the world, about 50.8 million in India⁶, 11 million in Brazil⁷, and 26 million people in U.S population⁸. Due to its high prevalence and potential deleterious effect on a patient's physical and psychological state, diabetes is a major medical concern⁹ 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 species¹⁰. 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 etc¹¹. 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 USA¹² 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.

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 mellitus¹³. In type 1 diabetes there is autoimmune destruction of β -cells which is triggered by the factors such as environmental and viral factors¹⁴. In type 2 diabetes β -cells destruction leads to decrease in insulin release and resistance to insulin develops due to obesity¹⁵. 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 tissue¹⁶. 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 damage¹⁷. Indirectly mitochondrial ROS produces oxidative stress which functions as a signalling molecule to activate stress sensitive pathways such as nuclear factor γ B (NF- κ B), janus kinases/ stress activated protein kinases (JNK/SAPK), and hexosamines. These stress sensitive pathway increases production of sorbitol, advance glycated end product (AGE), cytokines and prostanoids along with diacyl glycerol (DAG) which collectively results in insulin resistance and β -cell dysfunctioning¹⁸. 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 destruction¹⁹. 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 mellitus²⁰. Beyond above mention factors there are

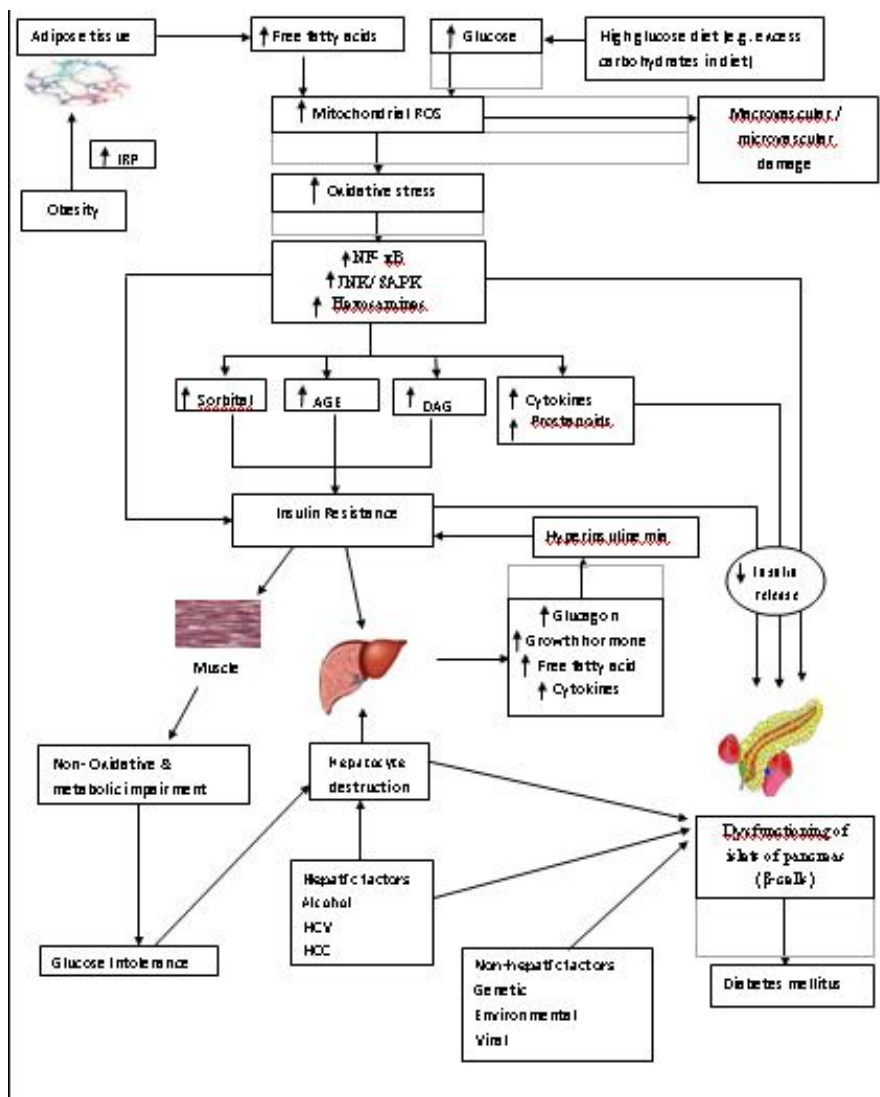
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some non-hepatic factors like genetic²¹, environmental and viral factors that are directly involved in the diabetes mellitus²².

Fig.1: Pathogenesis of diabetes.



IRP = Inflammation related protein, ROS = Reactive oxygen species, GAPDH= Glyceraldehyde-3-phosphate dehydrogenase, 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 below²³.

- 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-dioxuracil) is hydrophilic, unstable substance having half life about 1.5 min (at temp 37°C) and is longer at lower temperatures²⁴ 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 precautionally²⁵.

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 *Streptomyces achromogenes* (species of gram positive

bacteria that belongs to genus Streptomyces) and is used to induce both type 1 diabetes and type 2 diabetes at dose of 50mg/kg i.p for 3 days²⁶ and 80-100mg/kg (i.v, i.p or s.c) in neonatal rats for 10days²⁴. STZ can also be used in higher animals to induce diabetes given in table-1.

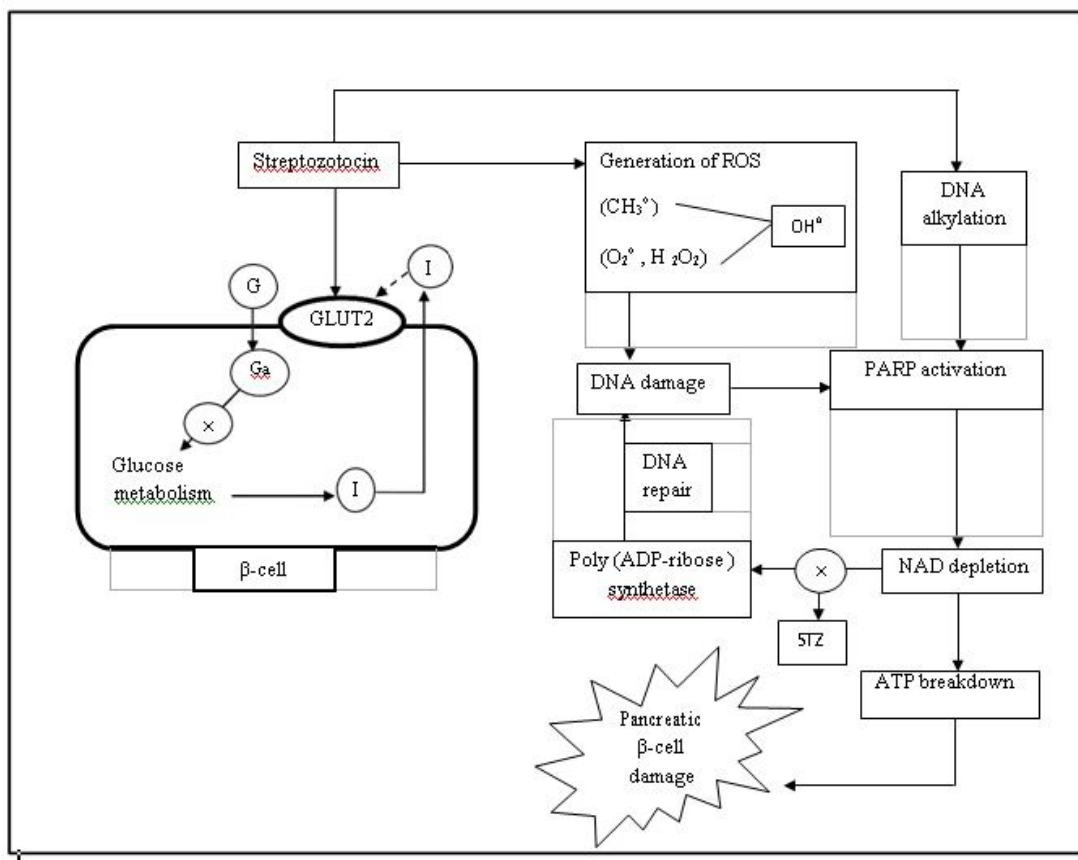
Table - 1: Streptozotocin induced diabetes mellitus in different experimental animal species

| 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^\bullet), oxygen radicals (O_2^\bullet), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^\bullet) that results in DNA damage³⁵ 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 damage²⁷.

Figure 2: Mechanism of action of Streptozotocin (STZ)



GLUT2= glucose transporter, G= extracellular glucose, Ga= Glucose receptor activated, I = insulin, OH^\bullet = hydroxyl radical, CH_3^\bullet = methyl radical, O_2^\bullet = 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 fragmentation³⁶.

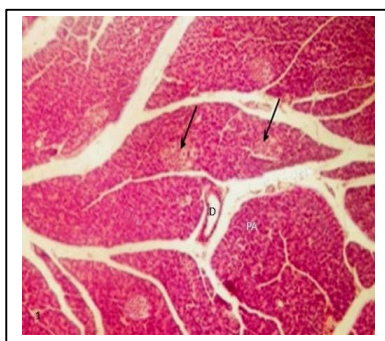


Fig.3: A Photomicrograph of rat pancreatic tissue of the control group showing normal lobular architecture. Islets of Langerhans (black arrows) seen surrounded by the pancreatic acini (PA). Notice the interlobular connective tissue (CT) and the interlobular duct (D).

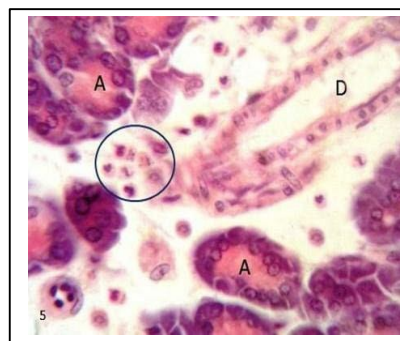


Fig. 4: A photomicrograph of rat pancreatic tissue of the diabetic group showing inflammatory cells (blue circle) surrounding duct (D) and between acini (A).

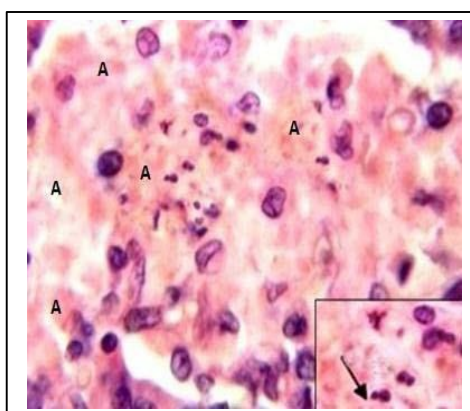


Fig.5: A photomicrograph of rat pancreatic tissue of the diabetic group showing inflammatory cells (blue circle) surrounding duct (D) and between acini (A).

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 liver³⁷, the δ -cell secrete somatostatin, 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 apoptosis³⁸.

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 metabolism³⁸. 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 hyperglycemia⁴¹.

2.1.3.3 Role of Thyroid hormone (T₃) in diabetes mellitus

Thyroid hormones are widely known for their ability to influence various cellular processes such as mitogenesis and differentiation, which are considered good target for counteracting the diabetes mellitus⁴². Preventive role of T₃ 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}.

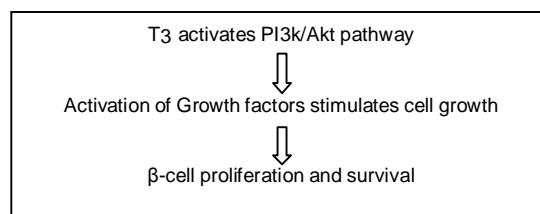


Fig.6: Preventive role of T₃ in Diabetes mellitus

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 -cells⁴⁵ or by initiation of an autoimmune response to β -cells²³.

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 circulation ⁴⁶ |
| 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 insulin⁵² and several other factors such as genetic factor, insulin purity, insulin aggregation involved in the generation of insulin antibodies⁵³. 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 antibodies⁵⁴.

2.1.6 Other Diabetogenic Compounds

2.1.6.1 Dithizone

Dithizone is sulphur containing organic compound (8-(p-toluene sulfonylamino) quinoline) a good ligand and forms complex with many metals such as lead and mercury⁵⁵. It is used to assess the purity of human pancreatic islet preparation that are used for transplantation in type 1 diabetic subjects⁵⁶. 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 them⁵⁷. Dithizone induces similar glycemic changes in rabbits, golden hamster and mice (triphasic fluctuations resulting in diabetes)

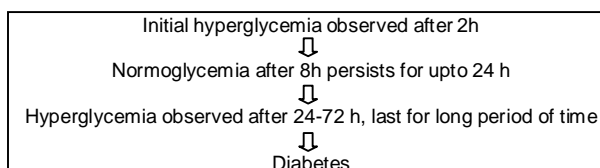


Fig. 7: Triphasic fluctuations in dithizone induced diabetes

2.1.6.2 Monosodium Glutamate

Monosodium glutamate (MSG) ingestion is known to increase plasma glutamate concentration. MSG infusion stimulates insulin secretion⁵⁸. 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) mouse⁵⁹.

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 defects⁶⁰. 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 | <ul style="list-style-type: none"> Associated with insulin deficiency and insulinitis due to autoimmune destruction of β- cell. Pancreatic islets subjected to an immune attack with T cells, B cells and macrophage recruited to insulinitis. 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 | <ul style="list-style-type: none"> Stable hyperglycemia in adult life span. Both insulin resistance and impaired insulin secretion are present. Associated with type 2 diabetes mellitus⁶¹ |
| Otsuka Long Evan Tokushima Fatty rat (OLETF) | 18 weeks | <ul style="list-style-type: none"> Associated with glucose intolerance⁶². Pancreatic islets undergo 3 stages of histological changes: <ol style="list-style-type: none"> Cellular infiltration (16-20 week old) Hyperplasia (20-40 week) Finally islet become fibrotic and replaced by connective tissue⁶³. Susceptibility of diabetes: M> F² |
| Zucker diabetic fatty rat (ZDF/ DRT-FA) | 6-11 weeks | <ul style="list-style-type: none"> 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 | <ul style="list-style-type: none"> Zucker (fa/fa) fatty or obese rat results from simple autosomal recessive (fa) gene on chromosome 5. Associated with hyperinsulinemia, hyperlipidemia and hypertensive⁶⁵ Impaired glucose tolerance Used for screening different insulin sensitizing and anti obesity agents⁶⁴ |
| James C Russel-LA (JCR: LA)- Corpulent rat | 10 weeks | <ul style="list-style-type: none"> Associated with hypertriglyceridemia and hyperinsulinemia with impaired glucose tolerance and also susceptible to vascular arteriosclerotic lesion⁶⁶ Used as research model for development of atherosclerotic and myocardial lesions in association with syndrome- X metabolic profile⁶⁷ |

| | | |
|--|------------|---|
| LEW.1ARI/- iddm 9rats | 8-9 weeks | <ul style="list-style-type: none"> Autoimmune model of diabetes Exhibits prediabetic period with islet infiltration in a week before animal become hyperglycemic⁶⁸. Animal survives after the onset of overt diabetes and can be used to study diabetic complications⁶⁹. Susceptibility of diabetes: M= F²⁴ |
| KDP (Komeda diabetes prone) rats | 8-16 weeks | <ul style="list-style-type: none"> In non lymphopenic substrain, frequency of diabetes is 70% and have mild to severe insulinitis 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 rats⁶⁰. |
| Cohen diabetic rat (CDR) | 6 weeks | <ul style="list-style-type: none"> Genetically derived model of diet induced type 2 diabetes. Sensitive group developed β- cell dysfunctioning and reduced insulin secretion with insulin resistance⁷⁰ |
| ESS rat | 8 weeks | <ul style="list-style-type: none"> 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 rats⁷¹. |

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 | <ul style="list-style-type: none"> Insulinitis at age 4-5 week old, followed by subclinical β-cell destruction⁷². Auto antibodies : GAD, ICA Susceptibility of diabetes: F> M⁷³ |
| Nagoya Shibata Yasuda (NSY) mice | 48 weeks | <ul style="list-style-type: none"> Spontaneous type 2 diabetic model Impaired insulin secretion. Susceptibility of diabetes: M> F⁷⁴ |
| Kuo Kondo (KK) mice | 8 weeks | <ul style="list-style-type: none"> 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 pancreas⁷⁵. |
| KK- AY/yellow KK obese mice | 5 weeks | <ul style="list-style-type: none"> 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. Extrapankreatic action of antidiabetic drugs such as glimepiride 23. |
| AKITA diabetic mice | 3-4 weeks | <ul style="list-style-type: none"> 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 deficient (Lepob/ob) mice | 2-4weeks | <ul style="list-style-type: none"> 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 | <ul style="list-style-type: none"> 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 (*Ctenomys talarum*).

Table 5: Other species with inherited diabetic symptoms

| Species | Characteristic Features |
|--|--|
| Spiny mouse (<i>Acomys cahirinus</i>) | <ul style="list-style-type: none"> Nocturnal, large light brown mice that weight 30 -50g and having fur bristles on their backs. Diabetes occurs in about 15% of the animals and accompanied by hyperplasia of endocrine pancreas⁷⁸. Characterized by obesity, mild hyperglycemia and hyperinsulinemia⁷⁹. |
| Sand rat (<i>Psammodromus obesus</i>) | <ul style="list-style-type: none"> 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 ketosis⁸⁰. |
| African hamster (<i>Mystromys albicaudatus</i>) | <ul style="list-style-type: none"> Characterized by hyperglycemia, glycosuria, ketonuria, polyuria, polyphagia, polydipsia, pancreatic lesion and β-cell death⁸¹. |
| TUCO-TUCO (<i>Ctenomys talarum</i>) | <ul style="list-style-type: none"> 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 hyperphagic⁸². |

2.3 Miscellaneous Models

2.3.1 Invertebrate Animal Model

Drosophila model used for understanding metabolism⁸³ 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 disease⁸⁴. 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 diet⁸⁵.

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

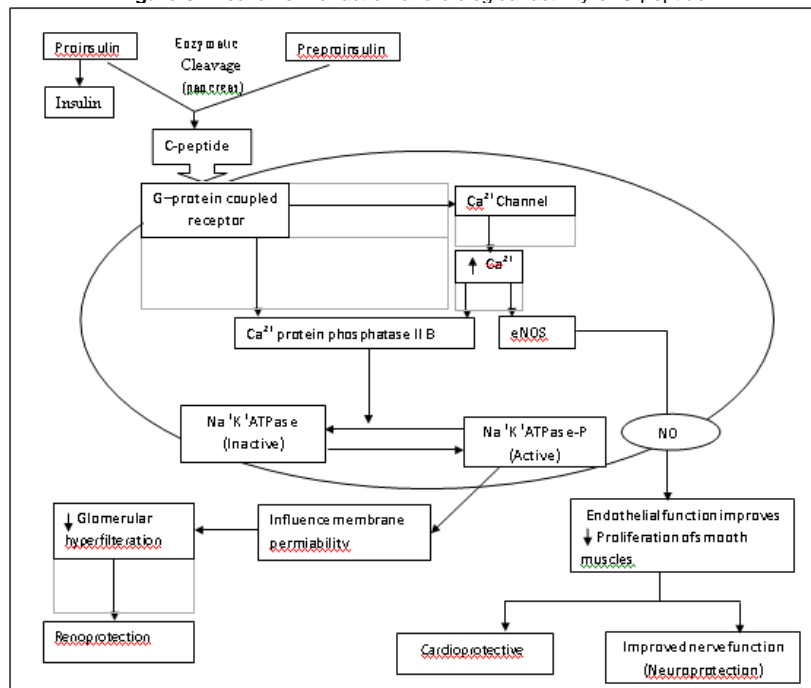
| Sl. No. | Plant name | Common name | Part used/ Type of extract | Observed result |
|---------|--|-----------------------|--|--|
| 1) | <i>Agele marmelos</i> (Rutaceae) | Holy fruit tree | Leaf/Aqueous extract | utilization of glucose ⁸⁸ |
| 2) | <i>Aloe vera</i> (Liliaceae) | Aloe | Whole plant/ Leaf pulp extract | maintain glucose homeostasis ⁸⁹ |
| 3) | <i>Annona squamosa</i> (Annonaceae) | Sugar apple | Leaf/ ethanolic leaf extract | blood glucose level ⁹⁰ |
| 4) | <i>Andrographis paniculata</i> (Acanthaceae) | King of bitter | Whole plant/ aqueous extract | prevent glucose absorption ⁹¹ |
| 5) | <i>Azadirachta indica</i> (Meliaceae) | Neem | Leaf/aqueous extract | hypoglycemic activity ⁹² |
| 6) | <i>Cassia auriculata</i> (leguminosae) | Tanner's cassia | Flower/ aqueous extract | enhanced utilization of glucose ⁹³ |
| 7) | <i>Caesalpinia bonducella</i> (Caesalpinioideae) | Chinese cinnamon | Seed/aqueous and ethanolic extract | release of insulin ⁹⁴ |
| 8) | <i>Citrullus colocynthis</i> (Cucurbitaceae) | Bitter apple | Seed/ aqueous extract | level of Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)and shows hypoglycemic activity ⁹² |
| 9) | <i>Casearia esculenta</i> (Flacourtiaceae) | Carilla | Root/aqueous extract | blood glucose level ⁹⁵ |
| 10) | <i>Catharanthus roseus</i> (Apocynaceae) | Madagascar periwinkle | Leaf and twigs/ ethanolic leaf extract | mobilization of glucose ⁹⁶ |
| 11) | <i>Eugenia jambolana</i> (Myrtaceae) | Indian black berry | Seed powder/ ethanolic seed extract | shows better glucose tolerance ⁶ |
| 12) | <i>Morus alba</i> (Moraceae) | White mulberry | Leaf/ aqueous extract | glucose uptake ⁹⁷ |
| 13) | <i>Mangifera indica</i> (Anacardiaceae) | Mango | Leaf/ aqueous extract | glucose absorption ⁹⁸ |
| 14) | <i>Ocimum sanctum</i> (Lamiaceae) | Holy Basil | Whole plant, leaf/ leaf powder extract | glucose level ⁹⁹ |
| 15) | <i>Punica granatum</i> (Punicaceae) | Pomegranate | Flower/ methanolic seed and flower extract | 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) | <i>Anacardium occidentale</i> (Anacardiaceae) | Cashew plant | Stems and Bark/ methanolic extract | blood glucose level ¹⁰¹ |
| 2) | <i>Amaranthus cruentus</i> (Amaranthaceae) | Amarath | Seeds/aqueous extract | malondialdehyde level in plasma ¹⁰² |
| 3) | <i>Andrographis paniculata</i> (Acanthaceae) | Chieretta | Whole plant, Leaf and Stem/ethanolic extract | antidiabetic and antioxidant activity ¹⁰³ |
| 4) | <i>Allium sativum</i> (Liliaceae) | Garlic | Bulbs/ Homogenates | improve insulin sensitivity ¹⁰⁴ |
| 5) | <i>Azadirachta indica</i> (Maliaceae) | Neem | Leaves/ aqueous extract | improve glucose intolerance ¹⁰⁵ |
| 6) | <i>Fagopyrum tataricum</i> (Polygonaceae) | Buck wheat | Roots/ powder ethanolic extract | glucose and triglyceride level ¹⁰⁶ |
| 7) | <i>Ficus exasperata</i> (Moraceae) | Vahl | Leaves aqueous extraction | ameliorated glucose tolerance ¹⁰⁷ |
| 8) | <i>Gymnema sylvestre</i> (Asclepiadaceae) | Gurmar | Leaves/ aqueous extraction | glucose and cholesterol level ¹⁰⁸ |
| 9) | <i>Ibervillea sonorae</i> (Cucurbitaceae) | Wareke | Roots/powder extract | glucose level ¹⁰⁹ |
| 10) | <i>Salacia chinensis</i> (Celastraceae) | Modhupal | Leaves/aqueous extract | serum glucose level ¹⁰⁸ |
| 11) | <i>Syzygium cumini</i> (Myrtaceae) | Jamun | Roots/powder extract | serum glucose level ¹⁰⁸ |
| 12) | <i>Tamarindus indica</i> (Caesalpinaceae) | Tamarind | Seeds/aqueous extract | Low density lipoprotein (LDL) and high density lipoprotein (HDL) level ¹¹⁰ |
| 13) | <i>Trigonella foenum graecum</i> (Leguminosae) | Fenugreek | Seeds/aqueous extract | improved insulin sensitivity ¹¹¹ |
| 14) | <i>Vitis vinifera</i> (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) | <i>Glycyrrhiza glabra</i> (Fabaceae) | Liquorice | Roots/ethanolic extract | LDL level and HDL level ¹¹³ |
| 2) | <i>Stevia rebaudiana</i> (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.

Ca²⁺ = 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, cholesterol 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 diabetes¹¹⁵.

3.1 C-Peptide

C-peptide is a polypeptide with molecular weight (MW) 3600, containing 31 amino 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 effects¹¹⁶.

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 pancreas¹¹⁶. C-peptide acts through G-protein coupled receptor to activate calcium-dependent signalling pathway¹¹⁷. This calcium signalling pathway is thought to activate the inactive form of Na⁺ K⁺ ATPase enzyme through phosphorylation of Phosphatase II B Ca²⁺ protein, ultimately influence the membrane permeability and leads to decreased glomerular hyperfiltration 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 hyperfiltration and reduce urinary albumin excretion¹¹⁹, enhanced Na⁺, K⁺-ATPase activity in neuropathy¹²⁰ and prevents vascular dysfunctioning¹¹⁸ in experimental and type 1 diabetes¹¹⁹.

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 cells¹²⁰. GHb in blood is the only protein that widely used to monitor glycemic control¹²². According to Americans Diabetic association, in normal adults HbA1c 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 retinopathy¹²³.

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