INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)
Platform for Pharmaceutical Researches & Innovative Ideas
www.ijprd.com

ANIMAL MODELS FOR TYPE-2 DIABETES MELLITUS: A REVIEW

S. N. Manjula*[1],
Sweety Javia[1], K. N. Pramod Chakravarthy[1], M. Madhu Raghav[1], Mina Basirian[1], K. Mrurhunjaya[1]

[1]J.S.S. College of Pharmacy, Department of Pharmacology, Sri Shivrathreeshwara Nagara, and Mysore-15

ABSTRACT

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide thus the disease constitutes a major health problem. Animal models have extremely contributed to the study of diabetes mellitus, a metabolic disease with abnormal glucose homeostasis, due to some defect in the secretion or the action of insulin. They give researchers the opportunity to control in vivo the genetic and environmental factors that may influence the development of the disease and establishment of its complications, and thus gain new information about its handling and treatment in humans. Most experiments are carried out on rodents, even though other species with human-like biological characteristics are also used. Animal models develop diabetes either spontaneously or by using chemical, surgical, genetic or other techniques, and depict many clinical features or related phenotypes of the disease. In this review, an overview of the most commonly used animal models of diabetes are provided, highlighting the advantages and limitations of each model, and discussing their usefulness and contribution in the field of diabetes research.

Correspondence to Author

S. N. Manjula

J.S.S. College of Pharmacy, Department of Pharmacology, Sri Shivrathreeshwara Nagara, and Mysore-15

Email: snm.manjula@gmail.com

Key words: animal model, diabetes mellitus, hyperglycemia, Glucose homeostasis, insulin resistance

INTRODUCTION

Diabetes mellitus is characterized by hyperglycemia, hypercholesterolemia, and hypertriglyceridemia, resulting from defects in insulin secretion or reduced sensitivity of the tissue to insulin (insulin resistance) and/or combination of both[1.] The worldwide survey reported that the diabetes is affecting nearly 10% of the population[2]. It is the third leading cause of death (after heart disease and cancer) in many developed countries.

Two major types of diabetes, one associated with insulin deficiency called Type-I or insulin dependent diabetes mellitus (IDDM) and other one is associated with insulin resistance called Type-2 or Non insulin dependent diabetes mellitus (NIDDM). Type-I is associated with a

specific and complete loss of pancreatic beta calls. Type-II is the most common type and associated with obesity, hyperinsulinemia and insulin resistance[3].

It is a serious endocrine syndrome with poor metabolic control and responsible for increased risk of cardiovascular diseases including atherosclerosis, renal failure, blindness or diabetic cataract worldwide [4].

Moreover, diabetes research in humans is impeded by obvious ethical considerations, because provocation of disease is strictly impermissible in man. Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes.[5]

Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic considerations. The large number of different models developed for different traits and insufficient characterization of some models make it difficult to choose the right model for a given study (including pharmacological screening) and at times leading to misinterpretation of data or even to the wrong conclusions. It is also very important to select appropriate animal model for the screening of new chemical entities (NCEs) and other therapeutic modalities for the treatment of type 2 diabetes.[6]

Oral glucose loading animal model:

This method is often referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is transiently increased with no damage to the pancreas. In the clinical setting, it is known as Glucose tolerance testing (GTT): a standard procedure often used for the diagnosis of border line diabetes. In this procedure, the animals are fasted overnight, treatment with test drug is given then oral glucose load (1- 2.5 g/kg body weight) is given and blood glucose level is monitored over a period of time. Glucose clearance period is observed. [7]

Disadvantage:

This method found to produce a widely fluctuating level in result.

This method used only for preliminary screening of newer antidiabetic drugs.

Normoglycemic animal model:

Normal healthy animals can be used for testing potential of oral hypoglycemic agents. This is still a valid screening method which is often used in addition to diabetic animal models (Williamson *et al*., 1996). This method allows for the effect of the drug to be tested in the animal with an intact pancreatic activity. For the evaluation of hypoglycemic agents, the animals have free access to normal diet till the experiment. Test drug will be administered. Blood is withdrawn at 1,2,3,4,5,48 and 72 hour after treatment. Blood glucose level is determined. In normoglycemic animal model mostly rat, rabbit, dog will be used.[8]

Disadvantage of normoglycaemic animal model:

A result obtained by this method is not accurate.

Chemically induced diabetes:

Chemical which induces diabetes is called as diabetogenic agent. Chemical agents which produce diabetes can be classified into three categories, and include agents that: Specifically damage β- cell, cause temporary inhibition of insulin production and/ or secretion and diminish the metabolic efficacy of insulin in target tissue.

Alloxan induced diabetes:

Alloxan may be called mesozalylurea, mesoxalylcarbamide 2, 4, 5, 6- tetraoxohexa hydropyrimidine or pyimidinetetrone. Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3.

Species	Dose	Route
Rat	40-200mg/kg	i.v or i.p.
Mice	50-200 mg/kg	i.v or i.p.
Rabbit	100-150 mg/kg	i.v.
Dog	50-75 mg/kg	i.v.

Mechanism of diabetogenic action of alloxan: Oxidative free radical (OFR) has been implicated in the etiopathogenesis of clinical diabetes mellitus. After injection of alloxan in rats, it is

selectively taken up by islets and hepatocytes. The liver has a very high concentration of OFR scavenging Enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxides (GPX), which are by comparison low in the islet cells. After administration alloxan get converted in to dialuric acid in islets which is unstable and is oxidized back to Alloxan, a reaction accompanied by reduction of oxygen to the OFR, O_2^- and $H_2O_2^-$, the later, through a Fenton type reaction in the presence of transition metals generates the highly toxic OFR, OH^-. Increased production of OFR in the islets, together with inadequate defense makes the β-islet cells susceptible to alloxan. Alloxan induces membrane lipid peroxidation and extensive DNA strand breakage in these cells. In normal non-fasted animals the blood glucose level after Alloxan injection fluctuates in a triphasic pattern. Alloxan acts by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyperglycemia and ketosis. [9]

Triphasic response of Alloxan:

1. Early hyperglycemia of short duration (about 1-4 hr) due to a sudden short lasting decrease or cessation of insulin release and direct glycogenolytic effects on the liver.
2. Hyperglycemia phase lasting up to 48 hrs and often resulting in convulsion and death
3. Chronic diabetic phase consequence of insulin lack histologically only a few β- cells if any are detectable in animals with fully developed alloxan diabetes. Exogenous insulin readily restores normal blood glucose levels. [10]

Disadvantage of Alloxan as diabetogenic agent:

- Hyperglycemia develops primarily by direct cytotoxic action on the beta cells and insulin deficiency rather than consequence of insulin resistance.
- Variability of results on development of hyperglycemia is perhaps high.
- Mortality of Alloxan induced diabetic animal is more (37%)
- Diabetes induced by alloxan is less stable and time reversal.
- Causes ketosis in animals due to free fatty acid generation.

Goldthioglucose (GTG) obese diabetic mouse model:

Type 2 diabetes with obesity is induced in mice by Goldthioglucose (GTG) (150-350 mg/kg, i.p.) injection. Mice gradually develop obesity, hyperinsulinaemia, hyperglycemia, and insulin resistance over a period of 16- 20 wk after GTG injection. The GTG is transported in particular to the cells of ventromedial hypothalamus (VMH) and causes necrotic lesions, which subsequently is responsible for the development of hyperphagia and obesity. It also shows increased body lipid and hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreased glucose metabolism in muscle, abnormalities that are qualitatively similar to genetically obese mice (*ob/ob*). In addition, it exhibits many molecular defects in relation to insulin signaling pathways. [11]

Disadvantage of GTG as diabetogenic agent:

- It will take long time to induce insulin resistance;
- Variability of results on development of hyperglycemia is perhaps high.
- Number of mortality following GTG injection is extremely high which limits its use in diabetes research.
- Expensive

Streptozotocin (STZ) induced diabetes:

Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Type of diabetes and characteristics differ with the employed dose of STZ in different animal and species. Single diabetogenic dose of STZ (70-250mg/kg, body weight) has been demonstrated to induce complete destruction of beta cells in most species within 24 hour. [5]

STZ induces diabetes in almost all species. Diabetes can be induced by STZ either by either by single injection of STZ or by multiple low dose

injection of STZ. STZ is the most commonly used drug for induction of diabetes in rats. [12]

STZ diabetic animals are most widely used for screening the compounds including natural products for their insulinomimetic, insulinotropic and other hypoglycaemic/ antihyperglycaemic activities.

Initial hyperglycemia is observed by 1 hour after the STZ injection followed by hypoglycemia and again a hyperglycemia state at 48 hours, the elevated blood glucose level was observed by 48-72 hrs (peak, effect) and was maintained thereafter. [13]

Different mechanisms of action on the β-cells destruction by STZ have been proposed. The evidences are accumulating on the mechanisms associated with diabetogenecity of STZ. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Their main action is through free radical generation. Other report proposed that STZ exerts lethal damage by alkylation to the DNA. STZ also influences the Immune system by suppressing the T-cell function associated with atrophy of the thymus and peripheral lymphoid tissue. STZ also induces OFR induced lipid peroxidation and DNA strand breaking in pancreatic islet cells. STZ have been shown to significantly reduce islet cell SOD activity in rats and mice. [6]

The doses of STZ as diabetogens in different species:

Species	Dose	Route
Rat	35-65	i.v. or i.p.
Mice	100-200	i.v. or i.p.
Hamster	50	i.p.
Dog	20-30	i.v.
Pig	100-150	i.v.

Neonatal Streptozotocin induced diabetes rat model (nSTZ):

Single dose of STZ to the neonatal rat will induce Type-2 diabetes. Single dose of STZ 100 mg/kg i.p. to the one day old pup and 120 mg/kg i.p. to the two, three, or five day old pups induce the diabetes. The neonatal STZ rats are considered to be better tools for the elucidation of the mechanisms associated with regeneration of the beta cells, the functional exhaustion of the beta cells and the emergence of defects in insulin action. [14,15]

Nicotinamide-Streptozotocin (NAD-STZ) induced diabetic model:

The rats administered NAD (120 mg/kg, ip) 15 min before STZ (60 mg/kg, ip) has been shown to develop moderate and stable non-fasting hyperglycemia without any significant change in plasma insulin level. As NAD is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass producing type 2 diabetes. Therefore, this model is found to be an advantageous tool for investigation of insulinotropic agents in the treatment of type 2 diabetes. [16]

Disadvantage of STZ as diabetogenic agent:

- Mortality of STZ induced diabetic animal is 7%
- Variability of results on development of hyperglycemia is perhaps high.
- Hyperglycemia develops primarily by direct cytotoxic action on beta cells and insulin deficiency rather than consequence of insulin resistance.
- Guinea pig and rabbits are resistant to its diabetogenic action.

Differences between Alloxan and Streptozotocin:

Alloxan	Streptozotocin
Maximum blood glucose level during phase of acute hyperglycemia occurs within 45 min.	Maximum blood glucose level during phase of acute hyperglycemia occurs within 120 min.
Liver glycogen depletes faster Hypoglycemia less severe	Liver glycogen depletes slowly Hypoglycemia more severe
Mortality rate 37%	Mortality rate 8%

Dithizone induced diabetes:

Organic agents **(**8-(p-toluene-sulfonylamino) quinoline**)** (TSQ) react with zinc in islets of Langerhans causing destruction of islet cells and producing diabetes. Dithizone injection at a dose level of 50-200 mg/ kg will produce triphasic glycemic reaction. Initial hyperglycemia will be observed after 2h & normoglycemia after 8h, which persist for upto 24 h. again hyperglycemia, is observed after 24-72 h which last for long period of time. [16, 17]

Disadvantage of Dithizone as diabetogenic action:

- Variability of results on development of hyperglycemia is perhaps high.
- Mortality is more.

Surgically induced diabetes:

This method consists of complete or partial removal of pancreas in animals used for the induction of Type 1 or type 2 diabetes. Few researchers have employed this model to explore effects of natural products with animal species such as rats, pigs, dogs and primates. [18, 19, 20] This method avoids cytotoxic effects of chemical diabetogens on other body organs and Resembles human type 2 diabetes due to reduced islet beta cell mass.

Partial pancreatectomy in animals performed as 70 or 90 per cent (usually 90%) dissection of pancreas has been reported in various animal species mostly in dogs, pigs, rabbit and also Rats. Depending on the amount of intact pancreatic cells, diabetes may range in duration from few days to several months. [21] This experimental design permits to evaluate if the compound has some effect upon both resistance and secretion of insulin.

Total removal of pancreas results in an insulin-dependent form of diabetes, and insulin therapy is required to maintain experimental animals. The portion of the pancreas usually left intact following a subtotal pancreatic resection is typically the anterior lobe or portion thereof.

The use of pancreatectomy in combination with chemical agents, such as Alloxan and STZ, produces a stable form of diabetes mellitus in animals. The combination therapy reduces the organ damage associated with chemical induction and minimizes the interventions, such as enzyme supplementation, necessary to maintain a pancreatectomized animal. [22] Limitation to this technique include high level of technical expertise and adequate surgical room environment, major surgery and high risk of animal infection, adequate post-operative analgesia and antibiotic administration, supplementation with pancreatic enzymes to prevent malabsorption and loss of pancreatic counter regulatory response to hypoglycemia.

Disadvantage of surgically induced diabetic animal model:

- Surgical removal of pancreas results in loss of α- and δ- cells in addition to β-cells. This causes loss of counter-regulatory hormones, glucagon and somatostatin.
- The total resection of the pancreas in rat is very difficult to achieve and the development and severity of the diabetic state appear to be strain specific.
- Involvement of cumbersome technical and post operative procedures.
- Occurrence of some other digestive problems (as a result of part of excision of exocrine portion (deficiency of amylase enzyme)

- Mortality is comparatively higher.

Diabetes induced by viral agent:

Viruses are thought to be one of the agents for IDDM. Viruses produce diabetes mellitus by: Destroying and infecting pancreatic beta cells. A less infecting or cytologic variant producing a comparable damage by eliciting immune auto reactivity to the β-cells. [23]

Various human viruses used for inducing diabetes include RNA picornoviruses, Coxackie B4, encephalomylocarditis (EMC-D and M variants), Mengo-2T, reovirus, and lymphocytic choriomeningitis. [24]

Primary isolates of these human pathogenic agents are not pancreatotrophic or lytic to mouse beta cells and must be adapted for growth either by inoculation into suckling mice, or by passage in cultured mouse beta cells.[25]

Infection of mice with the M variant of encephalomyocarditis (EMC) virus results in beta cell damage and a clinical picture characterized by hyperglycemia, glycosuria, polydipsia, polyphagia, and hypoinsulinamia. Adult male ICR Swiss mice are susceptible to the diabetogenic effect of the D-variant of encephalomyocarditis virus in contrast to adult CH/HCT male mice, which are relatively resistant. [26,27]

Coxsackie B4 virus is strongly associated with the development of insulin-dependent diabetes mellitus in humans and shares sequence similarity with the islet auto antigen glutamic acid decarboxylase. [28]

Susceptible mouse strain:

Virus	Susceptible mouse strain
EMC-D or M variant	SJL/L, SWR/J, DBA/1J, DBA/2J,BALB/c
Kilham rat virus	BB-DR
Reo	SJLj
CB4	SJL/J
Mengo-2T	SJL, C57BL/6J, CBA/J, C3H/HCJ, CE/J AKR/J

Disadvantage of virally induced diabetes:

- A virus produces systemic effect, not directly affecting the β-cells.
- Mortality is comparatively higher.
- Highly sophisticated and costly procedure for induction and maintenance.

Hormone-induced diabetes mellitus:

Dexamethasone induced diabetes:

Dexamethasone, steroid possessing immunosuppressant action, which causes an autoimmune reaction in the islets and produces diabetes. Long acting glucocorticoid used to produce NIDDM. Dexamethasone, the GLUT1 protein expression level was decreased, which possibly caused decreased basal glucose uptake. On the other hand, dexamethasone treatment did not alter the amount of GLUT4 protein in total cell lysates but decreased the insulin-stimulated GLUT4 translocation to the plasma membrane, which possibly caused decreased insulin-stimulated glucose uptake. Insulin resistance may result from decreased muscle blood flow, impaired cellular glucose transport, or intracellular deficits of glucose metabolism. Dexamethasone at a dose level of 2-5 mg/kg body weight i.p. twice a day in rat over a number of days produces NIDDM. [28,29]

Disadvantage of hormonal induced diabetes:

- Variability of results on development of hyperglycemia is perhaps high.
- Induction rate is less comparatively.

Genetically induced diabetic animal model:

Spontaneously diabetic animals of type 2 diabetes may be obtained from the animals with one or several genetic mutations transmitted from generation to generation (*e.g. db/db* mice)
or by selected from non-diabetic out bred animals by repeated breeding over several generation [*e.g.*,BB rat, Tsumara Suzuki Obese Diabetes (TSOD) mouse]. These animals generally inherited diabetes either as single or multigene defects.

(*e.g.* KK mouse, db/db mouse, or Zucker fatty rat).

***Zukker Diabetic Fatty Rat* (ZDF):** These arose from the inbreeding of a substrain of fa/fa (leptin receptor−deficient) rats that exhibited hyperglycemia. Zucker diabetic fatty (ZDF) rat is associated with disruption of normal islet architecture, β-cell degranulation, and increased β-cell death. In this strain all animals develop obesity, insulin resistance and overt NIDDM between 7 and 10 weeks of age, by which time their average plasma glucose exceeds 22 mM. [31]

BB rat (Biobreeding rat): Biobreeding rat also known as the BB or BBDP rat is an inbred laboratory rat strain that spontaneously develops autoimmune Type 1 Diabetes. The BB rat is among the best models of insulin−dependent diabetes mellitus — with onset and pathogenesis closely resembling the human disease. One unusual feature is a severe T−cell lymphopenia, which appears to be inherited as a recessive trait controlled by a single gene. [32]

***db/db mouse*:** The *db/db* (diabetic) mouse (now relabeled as *leprdb*) is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KsJ strain originating from Bar Harbor, Maine .The mutation in this diabetic animal was traced to *db* gene, which encodes for the leptin receptors. These mice are spontaneously hyperphagic insulin oversecretors becoming obese, hyperglycaemic, hyperinsulinaemic and insulin resistant within first month of age and develop hypoinsulinaemia, hyperglycaemia later with a peak between 3-4 months of age. Animals then exhibit ketosis, progressive body weight loss and do not survive longer than 8-10 months. [33]

KK mouse: KK (Kuo Kondo) mouse is polygenic model of obesity and type 2 diabetes produced by selective inbreeding for the large body size in Japan, also named as Japanese KK mouse. These animals are hyperphagic, hyperinsulinaemic, insulin resistant and show moderate obesity by 2 months of age, which attains maximum at 4-5 months. Insulin resistance precedes the onset of obesity. The increase in pancreatic insulin content is associated with increase in number and size of pancreatic islets but histologically degranulation of beta cells and hypertrophy of islets are found. There is selective failure of insulin to suppress gluconeogenic pathway, while exerting its inductive effect on glycolysis and lipogenesis as seen in hepatic insulin resistance of *db/db* mouse. [34,35]

TSOD mouse: By selective breeding of obese male mice of ddY strain, Tsumara and Suzuki described the two inbred strains, one with obesity with increase in urinary glucose named TSOD (Tsumara Suzuki Obese Diabetes) and other without them (TSNO, Tsumara Suzuki Non Obese). TSOD mouse is of polygenic origin and characterized by polydipsia and polyuria at about 2 months old only in male mice followed by hyperglycaemia and hyperinsulinaemia.

Following these symptoms, obesity gradually develops until about 12 months. Pancreatic islets of TSOD male mice are found hypertrophic without any signs of insulitis or fibrous formation. It has been shown that the reduced insulin sensitivity in diabetic TSOD mice is due, at least in part, to the impaired glucose transporter (GLUT4) translocation by insulin in both skeletal muscle and adipocytes. [37,38, 39]

Knockout animals: Knockout animals are produced by using a genetic construct that will disrupt normal gene. Construct is developed, which contains DNA sequence homologues to the target gene but that are disrupted or contain a deletion. These are injected into embryonic stem cells (ES) and will undergo recombination with the normal gene, causing it to be 'knocked out'. These will implant into the mouse embryos and transferred to the oviducts of psudopregnant mice and allowed to develop to term. [40]

Effect of single gene or mutation on diabetes can be investigated *in vivo*

This approach has been used to produce a large number of animals to understand the pathogenesis of Type 1 and Type 2 diabetes. [41, 42]

Disadvantage of knock out diabetic animals:

- Expensive for regular screening method.
- Highly sophisticated and costly procedure for the production and maintenance.

Insulin Antibodies-induced diabetes:

Bovine insulin along with CFA to guinea pigs produces anti-insulin antibodies. i.v. injection of 0.25-1.0 ml guinea pig anti-serum to rats induces a dose dependent increase in blood glucose levels upto 300 mg/dl. This effect is due to neutralization of endogenous insulin by insulin antibodies. It persists as long as the antibodies are capable of reacting with insulin remaining in the circulation. Large doses and prolonged administration are accompanied by ketonemia, ketonuria, glycosuria and acidosis. [43]

Disadvantage of Insulin Antibodies-induced diabetes:

- Lower dose, the diabetic syndrome is reversible after few hours.
- Expensive.

REFERENCES

1. Mishra Akansha. Antihyperglycemic activity of six edible plants in validated animal models of diabetes mellitus. Indian J Sci Technol 2009; 2:80–6.
2. Kim Jong Dae et al. Anti-diabetic activity of SMK001, a poly herbal formula in streptozotocin induced diabetic rats: therapeutic study. Biol Pharm Bull 2006;29:477–82.
3. Porth CM, Pathology concepts of altered health state. 3rd ed. JB lippincott
4. Prasad SK et al. Antidiabetic activity of some herbal plants in streptozotocin induced diabetic albino rats. Pak J Nutr 2009;8:551–7.
5. K. Srinivasan & P. Ramarao. Animal models in type 2 diabetes research: An overview. Indian J Med Res 125, March 2007, pp 451-472
6. Chattopadhyay C.,et al .Animal models for exp. Diabetic mellitus .Indian J of Exp Biology 1997;1141-5.
7. Choi S.B, Park C.H, Choi M.K, Jun D.W, Park S. (2004). Improvement of insulin resistance and insulin secreation by water extracts of *Cordiceps militaris, phellinus linteus and paecilomyce tenuipes* in 90% pancreatectomized rats. J. Biotech. and Biochem. 68: 2257-2264.
8. Geisen K. Special Pharmacology of the new sulfonylurea glimpiride. Drug research 1988; 38: 1120-30
9. Battell ML, Yuen VG, Verma S, McNeil JH. Other models of type 1 diabetes. In: McNeil JH, editor. *Experimental models of diabetes*. Florida, USA: CRC Press LLC; 1999: 219-29.
10. Rerup CC. Dugs producing diabetes through damage of the insulin secreting cells. *Pharmacol Rev* 1970; *22* : 485-518.
11. Le Marchand Brustel Y, Jeanrenaud B, Freychet P. Insulin binding and effects in isolated soleus muscle of lean and obese mice. Am J Physiol 1978; *234* : E348-58.
12. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin. *Proc Soc Exp BiolMed* 1967; *126* : 201-5.
13. Bonner-Weir S, Trent DF, Weir GC. Responses of neonatal rat islets to Streptozotocin; Limited B cell regeneration and hyperglycemia. *Diabetes* 1981; *30:* 64-9.
14. Larsen MO, Wilken M, Gotfredsen CF, Carr RD, Svendsen O, Rolin B. Mild streptozotocin diabetes in the Gottingen minipig. A novel model of moderate insulin deficiency and diabetes. *Am J Physiol Endocrinol Metab* 2002; *282* : E1342-51.
15. D.K.Arulmozhi, A. Veeranjaneyulu, S.L. Bodhankar. Neonatal Streptozotocin induced rat model for Type-2 diabetes: A Glance. Indian J Pharmacology, August 2004, vol:36, 217-21
16. Pellegrino M, Christopher B, Michelle M. Gerard R. (1998). Development of a new model of type II diabetes in adult rats administered with streptozotocin and nicotinamide. Diabetes 47: 224-230
17. McNeil JH. *Experimental models of diabetes*. Florida, USA: CRC Press LLC; 1999.
18. Pederson RA. Non insulin dependent animal models of diabetes mellitus, Experimental models of diabetes. Florida, USA: CRC press LLC; 1999, 337-98
19. Rees DA, Alcolado JC. (2005). Animal models of diabetes mellitus.Diabetic Medicine 22:359-370.

20. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, *et al*. Experimental NIDDM: development of a new model in adult rats administered Streptozotocin and nicotinamide. *Diabetes* 1998; *47* : 224-9.
21. Masiello P. Animal models of type11 diabetes with reduced pancreatic β-cell mass. The international Journal of Biochemistry and Cell Biology 38;2006:873-893.
22. Duff GL, Murray EGD. The pathology of the pancreas in experimental diabetes mellitus. Am J Med Sci 1945; 210: 81-95
23. Bates SH, Jones RB, Bailey CJ. Insulin-like effect of pinitol. Br J Pharmacol 2000; *130* : 1944-8.
24. Szopa TM, Titchener PA, Portwood ND. Diabetes mellitus due to viruses-some recent developments. Diabetologia 1963; 36:687-95
25. Yoon JW. The role of viruses and environmental factors in the induction of diabetes. Current Top Microbiol Immunol 1990; 164:95-123
26. Craighead, J. E. and M.F. Mclane. Diabetes mellitus: Induction in mice by encephalomyocarditis virus. Science 1968; 162-913
27. Craighead, J.E. and J. Steinke. Diabetes mellitus- like syndrome in mice infected with encephalomyocarditis virus. Am J Pathol 1971; 63-119
28. Marc S. Horwitz, Linda M. Bradley, Judith Harbertson, Troy Krahl, Jae Lee & Nora Sarvennick. Diabetes induced by Coxsackie virus: Initiation by bystander damage and not molecular mimicry. Nature Medicine 1998; 4, 781-85
29. L Tappy, D Randin. Mechanism of dexamethasone induced insulin resistance in healthy human. Journal of clinical endocrinology and metabolism 1994; 1063-1069.
30. Ogawa A, Johnson JH, Ohneda M, et al, Roles of insulin resistance and beta-cell dysfunction in dexametasone- induced diabetes. J Clin Invest 1992; 90: 497-503
31. Kahn SE. The importance of the beta-cell in the pathogenesis of type 2 diabetes mellitus. *Am J Med* 108 (Suppl 6a):2S–8S, 2000
32. Markholst, H., Eastman, S., Wilson, D., Andreasen, B.E. & Lernmark, A. Diabetes segregates as a single locus in crosses between inbred BB rats prone or resistant to diabetes. *J. exp. Med.* **174**, 297−300 ,2001
33. Shafrir E. Diabetes in animals: Contribution to the understanding of diabetes by study of its etiopathology in animal models. In: Porte D, Sherwin RS, Baron A, editors. *Diabetes mellitus*. NewYork: McGraw-Hill; 2003 p. 231-55.
34. Reddi AS, Camerini-Davalos RA. Hereditary diabetes in the KK mouse: an overview. *Adv Exp Med Biol* 1988; *246* : 7-15.
35. Velasquez MT, Kimmel PL, Michaelis OE IV. Animal models of spontaneous diabetic kidney disease. *FASEB J* 1990; *4* : 2850-90.
36. Ibanez-Camacho R, Meckes-Lozaya M, Mellado-Campos V. The hypoglucemic effect of *Opuntia streptocanthe* studied in different animal experimental models. *J Ethnopharmacol* 1983; *7* : 175-81.
37. Iizuka S, Suzuki W, Tabuchi M, Nagata M, Imamura S, Kobayashi Y, *et al*. Diabetic complications in a new animal model (TSOD mouse) of spontaneous NIDDM with obesity. *Exp Anim* 2005; *54* : 71-83.
38. Miura T, Suzuki W, Ishihara E, Arai I, Ishida H, Seino Y, *et al*. Impairment of insulin-stimulated GLUT4 translocation in skeletal muscle and adipose tissue in the Tsumura Suzuki obese diabetic mouse: a new genetic animal model of type 2 diabetes. *Eur J Endocrinol* 2001; *45* : 785-90.
39. Pellegrino Masiello. Animal models of type 2 diabetes with reduced pancreatic beta-cell mass. The international J of Biochem & cell biology.2006;38:873-93
40. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabetic Med 2005;22:359-70
41. McIntosh CHS, Pederson RA. Non insulin dependent animal models of diabetes

mellitus.In: McNeil JH, editor. *Experimental models of diabetes*. Florida, USA: CRC Press LLC; 1999. p. 337-98.
42. Young DA, Ho RS, Bell PA, Cohen DK, McIntosh RH, Nadelson J. Inhibition of hepatic glucose production by STZ. Diabetes 1990; *39*: 1408-13.
43. Moloney PJ, Coval M. Antigenicity of insulin: diabetes induced by specific antibodies. Biochem J 1955; 59: 179-85
44. Antia B.S, Okokon J.E, Okon P.A. Hypoglyacaemic effect of aqueous leaf extract of *Persea Americana (*Mill*)* on alloxan induced diabetic rats. Indian J. pharmacol. 2005; 37:325-326.
45. Antonios Chatzigeorgiou, Antonios Halapas. The Use of Animal Models in the Study of Diabetes Mellitus. In vivo international journal of Experimental and clinical pathophysiology and drug research. February 2009; 23: 120-125.
46. Arison R.N, Feudale E.L. Induction of renal tumour by Streptozotocin in rats.1967; 214:1254-1255.
47. Atsuo Tahara, Akiko Matsuyama-Yokono, Ryosuke Nakano, Yuka Someya and Masayuki Shibasaki. Hypoglycemic Effects of Antidiabetic Drugs in Streptozotocin-Nicotinamide-Induced Mildly Diabetic and Streptozotocin-Induced Severely Diabetic Rats. Basic & Clinical Pharmacology & Toxicology*:* 1992*;*103*,* 560–568
48. Awai M, Narasaki M, Yamonoi Y, Seno S. Induction of diabetes in animals by parenteral administration of ferric nitrilotriacetate: A model of experimental hemochromatosis. Am. J. Pathol. 1979; (95)3: 663-673.
49. Babu V. Anti-hyperglycemic activity of Cassia Kleinii leaf extract in glucose fed normal rats and alloxan induced diabetic rats. Indian J Pharmacol 2002; 34:409-15
50. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. *Diabetes Care* 1989; *12* : 553-64.
51. Balamurugan, A.N., Miyamoto, M., Wang, W., Inoue K. and Tabata Y. (2003). Streptozotocin (STZ) used to induce diabetes in animal models 26: 102-103.
52. Battell ML, Yuen VG, Verma S, McNeil JH. Other models of type 1 diabetes. In: McNeil JH, editor. *Experimental models of diabetes*. Florida, USA: CRC Press LLC; 1999 p. 219-29.
53. Boucher, D. W and A.L.Notkins. virus induced diabetes mellitus. Company; 1990.
54. Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward WK (2004). Induction of type 1 diabetes mellitus in laboratory rats by use of alloxan; route of administration, pitfalls, and insulin treatment. Comprehensive Medicine 54: 252-257.
55. Fernandez-Alvarez J, Barbera A, Nadal B, Barcelo-Batllori S, Piquer S, Claret M. Stable and functional regeneration of pancreatic beta-cell population in nSTZrats treated with tungstate. *Diabetologia* 2004; *47:* 470-7.
56. Kasiviswanath R, Ramesh A, Kumar KE. Hypoglycemic and antihyperglycemic effect of *Gmelina asiatica* LINN. In normal and in alloxan induced diabetic rats. *Biol Pharm Bull* 2005; *28* : 729-32.
57. Katovitch MJ, Meldrum MJ, Vasselli JR. Beneficial effects of dietary acarbose in the streptozotocin induced diabetic rat. *Metabolism* 1991; *40* : 1275-82.
58. Kaufmann F, Rodriguez RR. Subtotal pancreactomy in five different rats strains incidence and course of development of diabetes. Diabetologia 1984; 27: 38-43
59. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, *et al*. Experimental NIDDM: development of a new model in adult rats administered Streptozotocin and nicotinamide. *Diabetes* 1998; *47* : 224-9.
60. Nuss JM, Wagman AS. Recent advances in therapeutic approaches to type 2 diabetes. *Ann Rep Med Chem* 2000; *35* : 211-20.
61. Ohno T, Kitoh J, Yamashita K, Ichikawa Y, Horio F, Terada M, *et al*. Toxin-induced IDDM (insulin dependent diabetes mellitus) in the musk shrew. *Life Sci* 1998; *63* : 455-62.

62. Ozturk Y, Atlan VM, Yildizoglu-Ari N. Effects of experimental diabetes and insulin on smooth muscle functions. *Pharmacol Rev* 1996; *48* : 69-112.
63. Paik S.G, Fleischer N, Shin S.I (1980) Insulin – dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory role of cell–mediated autoimmune process. Proc. Natl. Acad. Sci. 77(10): 6129-6133
64. Pellegrino M, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D. Development of a new model of type 2 diabetes in adult rats administered with Streptozotocin and nicotinamide. Diabetes1998; 47:224-229.
65. Rerup CC. Dugs producing diabetes through damage of the insulin secreting cells. *Pharmacol Rev* 1970; *22:* 485-518.
66. S.kumar, Animal models in diabetes mellitus. Indian J. Exp. Biol. 1997, 35, 1141-5.
67. Saltiel AR, Olefsky JM: Thiazolidinediones in the treatment of insulin resistance and type 2 diabetes. *Diabetes* **45**:1661–1669, 1996
68. Sasaki S, Nio Y, Hirahara N, Sato Y, Inoue Y, Iguchi C, *et al*. Intraperitoneally implanted artificial pancrease with transkaryotic beta-cells on microcarrier beads in a diffusion chamber improves hyperglycemia after 90% pancreatectomy in rats. *In Vivo* 2000; *14* : 535-41.
69. Shah Shweta, Bodhankar Subhash, Bhonde Ramesh, Mohan V.Combinative therapeutic approach for better blood sugar level control in alloxan diabetic mice. Int J Diabetes Metab 2006;14:104–5.
70. Sheng XQ, Huang KX, Xu HB. Influence of alloxan-induced diabetes and selenite treatment on blood glucose and glutathione levels in mice. *J Trace Elem Med Biol* 2005; *18* : 261-7.
71. Suzuki W, Iizuka S, Tabuchi M, Funo S, Yanagisawa T, Kimura M, *et al*. A new mouse model of spontaneous diabetes derived from ddY strain. *Exp Anim* 1999; *48* : 181-90.
72. Takasu N, Asawa T, Komiya I, Nagasawa Y, Yamada T. Alloxan induced DNA strand breaks in pancreatic islets. Journal of Biochemistry: 1991; 266: 2112- 2114.
73. Vogel HG, Wolfgay H. Vogel drug discovery and evaluation pharmacological assay. 2nd ed. J A Majors Company;1997.
74. Vogel HG, Wolfgay H. Vogel drug discovery and evaluation pharmacological assay.
75. Von Herrath M.G and Oldstone M.B. (1997). Interferongama is essential for destruction of beta cells and development of insulin-dependent diabetes mellitus. Journal of Experimental medicine. 185(3): 531-9.
76. Wellmann, K.F. and P. Brancato. Fine structure of pancreatic islets in mice infected with the M variant of the encephalomyocarditis virus. Diabetologia 1972;8-349
77. Williamson E.M, Okpoko D.T, Evans F.J (1996). Pharmacological methods in phytotherapy research. John Wiley and sons, Inc. Third Avenue, New York, USA. ISBN 0471 94216 2. pp. 155-167.

*****