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In vivo and *In vitro* Models for Biological Screening of Anti-Diabetic Drugs

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Abstract
The secondary metabolites have been traditionally used for diabetes treatment throughout the world; few of them have been validated by scientific criteria. The need for alternative strategies for the prevention and treatment of diabetes is growing rapidly as type-2 diabetes is accomplishment epidemic status in our society. The developing for novel bioactive compounds with antidiabetic properties to deal with the disease condition is still in progress. In the present review, we have attempted to bring together all the reported models for screening antidiabetic drugs using *In vivo* and *In vitro* models and the various *in vitro* techniques which include Inhibition of α-Amylase, α-Glucosidase activity and glucose uptake assay. Experimental induction of diabetes mellitus in animal models and *in vitro* techniques are essentials for the advancement of our knowledge, clear understanding of diabetogenic and for finding new therapy. The animal models and *in vitro* techniques are essentials for developing a new drug for the treatment of diabetes. The purpose of this review article is to describe the significance of animal models available for screening of antidiabetic activity.

Keywords
Antidiabetic Drugs, Streptozotocin, Alloxan, α-Amylase and α-Glucosidase.

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INTRODUCTION
Diabetes mellitus is one of the most common endocrine metabolic disorders which have a significant impact on the health, quality of life and life expectancy of patients as well as on the health care system. It characterized by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin (Vijayaraj and Sri Kumaran, 2018). It can be categorized as Type-1 diabetes [insulin dependent diabetes mellitus (IDDM)] and Type-2 diabetes [non- insulin dependent diabetes mellitus (NIDDM)]. The overall prevalence of diabetes is approximately 10% of the population, of which 90% is Type-2. The disease is characterized by hyperglycemia, hypercholesterolemia, and hypertriglyceridemia, resulting from defects in insulin secretion or reduced sensitivity of the tissue to insulin (insulin resistance) or combination of both. Characteristically, 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 (Vijayaraj *et al.,* 2016). According to the World Health Organization projections, the prevalence of diabetes is likely to increase by 35%. Currently, there are over 150 million diabetics worldwide and this is likely to increase to 300 million

or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world.

Despite the great interest in the development of new drugs to reduce the burden of this disease, the scientific community has raised interest to evaluate either raw or isolated natural products in experimental studies; few were tested clinically in humans. Experimental studies of diabetes in animal models and advanced *in vitro* techniques are essential for the improvement of knowledge and clear understanding of the pathology and pathogenesis and to find new therapy. Animal models of diabetes are therefore, greatly useful in biomedical studies because they offer the promise of new insights into human diabetes. Most of the available models are based on rodents because of their small size, shorter generation intervals and economic considerations. Experimental diabetes mellitus studied by several methods that include: chemical, surgical and genetic manipulations (Kumar *et al*., 2012). It is also very important to select appropriate animal model for the screening of new chemical entities (NCEs) and other therapeutic modalities for the treatment of diabetes (Chattopadhyay *et al.,* 1997). The main aim of the present review is to being together *in vitro* techniques and chemically induced *in vivo* animal models for carrying diabetes research.

Chemical agents which produce diabetes (diabetogenic agent) can be classified into three categories, and include agents that: specifically, damage ß- cell, cause temporary inhibition of insulin production and secretion and diminish the metabolic efficacy of insulin in target tissue. The following text is summarizes the models based on use of diabetogenic agents.

STREPTOZOTOCIN INDUCED DIABETIC MODEL

Streptozotocin (STZ) is a naturally occurring chemical it particularly produces toxic to the beta cells of the pancreas. It is used in medical research as an animal model for hyperglycemia and it is a chemotherapeutic agent which selectively destruct pancreatic islet (β) cell through the release of nitric oxide and also generates SOD anions which interacts with mitochondria that leads to diabetes and its associated complications (Papaccio *et al*., 2000; Szkudelski, 2001). The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves overproduction and decreased utilization of glucose by the tissues. The STZ alters the blood insulin and glucose concentrations. Two hours after injection, the hyperglycemia is due to the decreased blood insulin levels. Six hours later, hypoglycemia occurs due to the high levels of blood insulin. At last hyperglycemia develops and blood insulin levels drops. STZ impairs glucose oxidation (Bedoya *et al.,* 1996) and decreases insulin synthesis and release. It was observed that STZ at first abolished the B cell response to glucose. STZ restricts GLUT2 expression. STZ changes the DNA in pancreatic B cells (Morgan *et al.,* 1994). The B cell death is due to alkylation of DNA by STZ (Turk *et al.,* 1993). STZ-induced DNA damage activates poly ADPribosylation (Nukatsuka *et al*., 1990). The activation of poly ADP-ribosylation is of greater importance for the diabetogenicity of STZ than generation of free radicals and DNA damage.

ALLOXAN INDUCED DIABETIC MODEL

Alloxan is most widely used in experimental diabetic research. Alloxan produces selective necrosis of the beta cells of pancreas. The alloxan is administered by various routes like intravenous, intraperitoneal and subcutaneous. Alloxan is used for induction of diabetes in experimental animals such as mice, rats, rabbits and dogs. The routes and dose of alloxan required may vary depending upon the animal species (Iranloye et al., 2011). In alloxan, the hypoglycemic phase lasting for 30 min from the first minutes of alloxan administration. The hypoglycemic stage may be due to the stimulation of insulin release and high levels of plasma insulin levels. The mechanism at back of the hyperinsulinemia is due to the short term increase of ATP availability and glucokinase inhibition (Federiuk *et al., 2004*). The second phase is the increase in the blood glucose levels one hour after administration of alloxan, the plasma insulin concentration decreases. The pronounced hyperglycemia lasts for 2-4 hours is due to decrease plasma insulin concentrations. This may be due to inhibition of insulin secretion and beta cell toxicity (Kliber *et al.,* 1996). The third phase is hypoglycemic phase that last long for 4-8 hrs after administration of alloxan (Tasaka *et al.,* 1988). The treatment brings out a sudden rise in insulin secretion in the presence and absence of glucose. The insulin release occurs until the complete suppression of the islet response to glucose. Alloxan react with two sulfhydryl in the glucokinase resulting in disulfide bond and inactivation of the enzyme. The alloxan is reduced by GSH. Superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous ions. Fe3+ can also be reduced by alloxan radicals (Malaisse *et al.,* 1982). Szkudelski T (2001) reported the mechanism is the fragmentation of DNA in the beta cells exposed to alloxan. The disruption in intracellular calcium levels also contribute for the diabetogenic action of alloxan.

MONOSODIUM GLUTAMATE INDUCED DIABETIC MODEL

Monosodium glutamate (MSG) cause increase in plasma glutamate concentration. MSG stimulates insulin release. Administration of MSG in mice resulted in obesity associated with hyperinsulinemia. After 29 weeks, level of blood glucose, total cholesterol and triglyceride levels were increased (Kim *et al.,* 1997).

DITHIZONE INDUCED DIABETIC MODEL

Dithizone is an organosulfur compound, it has chelating property. Dithizone is used in induction of diabetes in experimental animals. In dithizonised diabetic animals, the levels of zinc, iron, and potassium in the blood were found to be higher than normal (Graham *et al.,* 2000). Dithizone permeates membranes and complex zinc inside liposomes, then release of protons, this enhances diabetogenicity.

GOLDTHIOGLUCOSE OBESE DIABETIC MODEL

Gold thioglucose (GTG) is a diabetogenic compound, which manifest obesity induced Type -2 diabetes. The intrapertonial administration GTG in experimental animal gradually develops obesity, hyperinsulinemia, hyperglycemia, insulin resistance for a period of 16- 20 weeks. The GTG is transported in particular to the cells and causes necrotic lesions, which is responsible for the development of hyperphagia and obesity. It also increases body lipid, hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreases glucose metabolism (Szopa *et al.,* 1993).

Table -1 Advantage and Disadvantage of Chemically Induced *In vivo* Antidiabetic activity

Chemically Induced *In vivo* Antidiabetic activity	
Advantage	**Disadvantage**
Selective loss of pancreatic beta cells (STZ /Alloxan) leaving other pancreatic alpha and delta cells intact.	Hyperglycemia develops primarily by direct cytotoxic action on the beta cells and insulin deficiency rather than consequence of insulin resistance.
Residual insulin secretion makes the animals live long without insulin treatment.	Variability of results on development of hyperglycemia is perhaps high.
Ketosis and resulting mortality is relatively less.	Diabetes induced by chemicals is mostly less stable and at times reversible because of the spontaneous regeneration of beta cells. Hence, care must be taken to assess the pancreatic beta cell function during long-term experiments.
Comparatively cheaper, easier to develop and maintain.	Chemical produce toxic actions on other body organs as well besides its cytotoxic action on beta cell.

SCREENING TECHNIQUES FOR *IN VITRO* ANTIDIABETIC ACTIVITY

ENZYME INHIBITION ASSAY FOR ANTI-DIABETIC ACTIVITY

α- AMYLASE INHIBITION ASSAY

The assay was performed with slight modification of a previously reported method (Sangeetha *et al.,* 2012). The test sample was prepared in dimethyl sulfoxide from 1 µg/mL^{-1} stock solution and the sample was added to a 0.5 mg/mL α-amylase solution and incubated for 10min at room temperature. 1.0% starch solution (500µL) was added and incubated at room temperature at 10min. After that 1 mL of dinitrosalicylic acid was added to the reaction mixture and heated in a boiling water bath for 5 minutes. After cooling, tested samples were diluted with 10 mL of distilled water. The absorbance was measured at 540nm. The percentage of enzyme inhibition activity of the bioactive fractions were calculated using the following formula shown in Equation (1).

$$\text{Inhibition activity (\%)} = \frac{\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Sample}}} \times 100 \quad \text{---------- (1)}$$

Where, Abs Sample is absorbance of test samples and Abs Control is the Absorbance of Control reactions (contains all reagents except the test sample). All the experiments were carried out in triplicates.

α -GLUCOSIDASE INHIBITION ASSAY

The assay was performed with slight modification of previously reported method (Ramachandran *et al.,* 2013). From 1 µg/mL^{-1} stock solution different concentrations of the test sample were prepared in dimethyl sulfoxide from 1 mg/mL stock solution and test sample were added to a 1 µg/mL^{-1} of α-glucosidase and then incubated for 5 minutes at

room temperature. Then 500uL of 37 mM maltose solution was added and incubated 30 minutes at room temperature. After that 1 mL of glucose peroxidase reagent was added to the reaction mixture and kept in room temperature for 15 minutes. Then 1 mL of Tris buffer was added to the mixture. The optical density was measured at 540 nm against the blank reagent. The percentage of enzyme inhibition activity of the bioactive fractions was calculated using the following formula shown in Equation (2).

$$\text{Inhibition activity (\%)} = \frac{\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Sample}}} \times 100 \quad \text{---------- (2)}$$

Where, Abs Sample is absorbance of test samples and Abs Control is the Absorbance of Control reactions (Contains all reagents except the test sample). All the experiment was carried out in triplicates.

GLUCOSE UPTAKE ASSAY

GLUCOSE UPTAKE ASSAY YEAST CELLS MODEL

Glucose uptake assay by yeast cells was performed according to Stranded principle and procedure (Cirillo *et al*., 1963). The yeast, *Saccharomyces* spp. suspended in distilled water was subjected to repeated centrifugation (3000 ×g, 5 min) until clear supernatant fluids were obtained and 10% (v/v) of the suspension was prepared in distilled water. Different concentrations of test sample were added to 1 mL of glucose solution (5 mM) and incubated together for 10 min at 37°C. The reaction started by adding 100µL of cell suspension followed by vortexing and further incubation at 37 °C for 60 min. Then tubes were centrifuged (2500 ×g, 5 min) and the amount of glucose was estimated in the supernatant. The percentage of inhibition activity was calculated using the following formula shown in Equation (3).

$$\text{Inhibition activity (\%)} = \frac{\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Sample}}} \times 100 \quad \text{---------- (3)}$$

Where, Abs Sample is absorbance of test samples and Abs Control is the Absorbance of Control reactions (Contains all reagents except the test sample). All the experiments were carried out in triplicates.

GLUCOSE UPTAKE ASSAY BY ADIPOCYTES CELL LINE

Adipose tissue is considered to have a link between obesity and Type 2 diabetes, elevated intracellular lipid concentrations and insulin resistance (Jarvill *et al.,* 2001). Insulin resistance either at the adipocyte or skeletal muscle levels contribute to hyperglycemia. Pathways related to insulin resistance may be studied in cell lines of adipocytes such as 3T3-L1 cells and rat L6 muscle engineered to over-express GLUT4.

CONCLUSION

In this review, we have discussed *In vitro,* and *In vivo* models used in diabetes research. Each model is essentials tools for investigating endocrine, metabolic, genetic changes and underlying mechanism of human diabetes. The animal models and *in vitro* techniques are essentials for developing a new drug for the treatment of diabetes. More animal models, software, advanced techniques have to be developed for advance research in diabetes and it is expected that more therapeutic alternatives may be available with future advances in diabetes research.

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