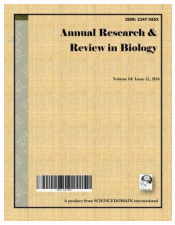

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A Review on Experimental Methods of Diabetic Research: Advantages and Limitations

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Authors' contributions

This work was carried out in collaboration between all authors. Author UZU wrote the first draft of the manuscript. Author MM initiated the idea, designed the review and reviewed the final draft. Author ABAB helped in literature search and reviewed the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Globally diabetes mellitus has become a medical problem with an increase in prevalence, morbidity and mortality. To date, it has no cure yet, however, medical care, healthy life style and exercise programme are provided to the patients to alleviate the diabetic symptoms and avoid complications. Animal model remains the best model for intensive diabetic research because of its flexibility, in order to investigate the possible curative and preventive measures for this life threatening metabolic disease. The purpose of this review is to update and compile all the documented procedures, methods or techniques in diabetic study using experimental animal models and highlight on their advantages and limitations. Some of the techniques used in studying diabetes mellitus are islets cells autoimmune antibodies, cytotoxic chemical substances, high sugar high fat diet, steroid therapy, surgical removal of the pancreatic tissue, transgenic animal model, normoglycemic animal and genetically modified animal model. Most of these techniques are currently applied in diabetic research while a few techniques are rarely used due to their nature, cost and time consuming. Hence, this review may help the researchers to select the most appropriate procedure to be used in their diabetic study using experimental animal model to suit their target and desired objective.

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1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome characterized with hyperglycemia, increased thirst, polyuria, polyphagia and weight loss. Gradually it alters and virtually affects all the systems in the body leading to an increase in acute, chronic as well as micro and macro vascular complications [1]. There were 382 million people in the world with diabetes in the year 2013 and it is projected to increase up to 592 million by 2035. Its effect on the patient socio-economy, physical and medical state has become a major concern globally [2]. To date, it can only be controlled by modern medicine, healthy life style and exercise despite much research is going on in this area [3,4]. Hence, there is a need for more research to explore all the possible causes or pathogenesis, routing screening and treatment of DM problems with scientific proof [5]. It is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately to come up with new preventive measures and therapies [6]. Animal model remains the best model for diabetic study at the moments for its flexibility, standardization and general ethical compliance.

The idea of the experimental induction of diabetes in animal models is very essential for the advancement of our knowledge and understanding of the various aspects of this diseases and ultimately finding new therapies, cure and final solution to this problem.

1.1 Classification

Diabetes is classified into four main categories:

- Type 1 diabetes which is due to beta-cell destruction or inadequate insulin secretion that usually leads to absolute insulin deficiency.
- Type 2 diabetes which is due to a progressive insulin receptor or insulin functional defect on the background of insulin resistance or gene mutation
- Gestational diabetes mellitus in which hyperglycemia develops only during pregnancy that is not clearly overt diabetes.
- Other types of diabetes are due to some associated causes such as genetic defects in beta cell normal physiology, genetic defects in insulin function, diseases affecting the islets or exocrine pancreas, beta cell cytotoxic chemicals or steroid therapy [1,7].

1.2 Diagnosis of Diabetes

The disease is usually diagnosed based on plasma glucose criteria, either the fasting blood sugar level of ≥126 mg/dL (≥7 mmol/L) or the two hour plasma glucose value of ≥200 mg/dL (≥11.1 mmol/L) after a 75 g glucose tolerance test. Recently, an International Expert Committee has added the glycated hemoglobin level of ≥6.5% as a third option to diagnose diabetes [1,7,8].

Animal models have provided suitable means for diabetes experimentation that would be impossible in humans. The purpose of this review is to narrate and update some of the methods used in achieving or monitoring experimental hyperglycemia or DM in animal models considering their advantages and limitations.

2. METHODS OF INDUCING DIABETES IN EXPERIMENTAL ANIMALS

In general, several techniques or procedures have been applied in achieving hyperglycemia and DM in animal study that reveals so many achievements and limitations. Some of these methods include the autoimmune reaction against the beta-cells of the pancreatic tissue (antigen-antibody body formation), surgical removal of the pancreatic tissue, in which case almost all the pancreatic tissues are safely needed to be removed [9], transgenic animal model, genetically modified diabetic animal model, steroid therapy, high sugar high fat diet, iron therapy, spontaneous diabetic species / model and normoglycemic animal [9,10]. The induction using cytotoxic chemicals such as alloxan and streptozotocin which selectively destroy pancreatic islets cells or more specific beta-cells are also being used [11,12]. These methods are designed based on the etiological condition resulting in either type 1 or type 2 DM depending on the mechanism in which hyperglycemia is achieved.

2.1 Autoimmune Mediated Islets Cells Destruction

This method includes the use of some pathogenic organism that can stimulate autoimmune response against the pancreatic tissue antigens leading to formation of antibodies against beta cells in which 70% of animals achieve DM [13]. This is developed by administering a unique viral protein, viral gene (eg; Encephalomyocarditis virus, Kilham rat virus) or pathogen into the experimental animals (mice, rat) inducing autoimmune responses against pancreatic beta cells, which leads to the formation and release of cytokines (IL-1 beta, IL-6, TNF-alpha) and impaired insulin functions [14]. This in turn, can cause morphological damage of islets cells leading to decreased glucose oxidation and decreased proinsulin biosynthesis, targeting to inactivate normal insulin functions to achieve DM within one week in mice [15]. The researcher has found that mango or encephalomyocarditis virus induces Types 1 DM in mice by destroying beta cells within 3-4 days [16]. This procedure is new and rarely used as it is tedious and requires expensive equipment and skills.

2.2 Surgical Approach

Removal of the pancreatic tissue (pancreatectomy) is very effective in which 100% of animals achieve DM. However, in order to achieve hyperglycemia, total or partial removal of the pancreatic tissue is needed by laparotomy [17]. The islets of Langerhans in the pancreas contain beta-cells which are responsible for synthesis of a polypeptide hormone known as insulin, a key hormone for glucose metabolism. Removal of pancreatic tissue to certain level will definitely cause hyperglycemia. Metabolic changes leading to type 1 DM will fully develop within 19.3 days after pancreatectomy in cats [18]. There are many limitations to this procedure, as it involves surgery and post-operative management. It is also time consuming and is restricted to only insulin dependent metabolic problems associated with the decrease in beta cell mass [19]. The induction of this Type 1 DM has also been suggested in pig [20]. In general, the above techniques are effective but rarely used in most diabetic research due to its nature.

2.3 Transgenic Genetically Modified Diabetic Animal Model

Genetic animal model of DM is genetically produced to under express (knockout) the protein (gene) responsible for carbohydrate metabolism in order to have mouse offspring with a sustained elevated blood glucose level [21]. The animals (rats) will develop impaired pancreatic beta-cells activity with subsequent reduced insulin secretion and function making this model as a good model of type 1 diabetes. This mutant protein (gene) will then appear as an antigen against the pancreatic tissue as its targeted cells and causes pancreatic islets cells destruction through antigen-antibody reaction and complex formation. However, the success rate of developing DM is 60% [22]. The technique is just emerging as new procedure of diabetes study in animal model and applicable for type 1 diabetic research only. However, it is not commonly used due to its nature which needs expertise, expensive and time consuming.

2.4 Steroids Therapy

Steroid such as prednisolone is cholesterol derivative that has cholesterol like structure with anti-inflammatory activity and can cause sodium and water retention [23,24]. This chemical is used to cause hyperglycemia by decreasing the sensitivity of insulin receptor to insulin and can also antagonise the peripheral action of insulin leading to altered immune activities, increased plasma glucose concentration and hyperglycemia detected by poor glucose tolerance test [25] and applicable to animal models of diabetes research such as rat [23], guinea pig [24] and rabbit [25]. This model is used for type 2 diabetes research and metabolic syndrome [26]. The maximum hyperglycemia (200 mg/dL) is achieved in all rabbits (100% success rate) within 4^{th} to 6^{th} day after the administration of hydrocortisone acetate intramuscularly at 5 mg/kg daily [25]. However, the steroid administration may also lead to hypertension and immunosuppression [24]. The technique is hardly used due to its systemic side effect, time consuming and less reliability.

2.5 High Sugar High Fat Diet

Chronic exposure to high sugar high fat diet is associated with adiposity gain and alterations in metabolic biomarkers in baboons, transient increase in blood glucose level with no damage

to the pancreas or liver are observed following eight week exposure. Persistent hyperglycemia will lead to Type 2 DM in experimental animals such as non-human primates Baboon (*Papio hamadryas sp*) due to chronic poor glucose tolerance. This in turn will trigger a progressive loss of pancreatic beta-cells differentiation in an animal model as well as reduced insulin sensitivity to its receptors over a period of time (after 8 weeks of dietary exposures) leading to symptoms of diabetes mellitus (physiological hyperglycemia) which may turn to normal after some time. All the animals are found to develop postprandial hyperglycemia [27]. However, this technique is suitable for a short time diabetic or acute hyperglycemic research.

2.6 Hemochromatosis by Iron Therapy

This method involves intraperitoneal administration of ferric nitrilotriacetate daily for 60 days in rats (6 mg/kg) and rabbits (10 mg/kg). All animals successfully develops DM. Hyperglycemia occurs as a result of inflammatory damage and blockage to insulin secreting beta-cells due to iron deposit on islets of pancreas, leading to a reduction in secretion and action of insulin [28]. This method is a good model of type 1 DM but rarely used due to time consuming, easily reversible and danger of organ damage or toxicity.

2.7 Spontaneous Diabetic Species/Model

This species is a special group of animal models which is having the potentials of being obese and developing insulin resistance, through the decreased sensitivity to insulin by its receptors [29]. They are developed from a selective breeding over many generations of poor glucose tolerant and susceptibility to type 1 and 2 diabetes in apparently non diabetic rats. One of the examples is "Goto-kakizaki rat. Hyperglycemia develops between the age of 7 to 8 weeks and they are generally a good animal model for metabolic syndrome study. Animals have to be bred for a period of time (weeks to months) to achieve hyperglycemia. These animals exhibit impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) test, associated with a decrease in the number and size of islets of Langerhans [30]. However, these models are also associated with a tendency to develop obesity, hypertension and hypercholesterolemia in most cases whereby they are used in metabolic and diabetes research [31].

2.8 Normal Animal Model (Normoglycemic Animal)

This animal model is a normal healthy normoglycemic animal used in the experiment to test the potential effect of substance or agent on glucose metabolism. This method is commonly used in addition to diabetic animal models in diabetes research as it allows comparison and analysis of some metabolic parameters in normal and altered pancreatic functions [32]. This will provide adequate information on either hypoglycemic or hyperglycemic properties of the substance of the study and also highlight on the substance potency. These groups are mainly used as a control group in experiment or to investigate for anti diabetic activity of a substance in an experimental study using animal such as rats [33].

2.9 Cytotoxic Chemicals

Cytotoxic chemicals such as alloxan and streptozotocin are commonly used in diabetes study as they cause damage of the pancreatic tissue leading to hyperglycemia.

2.9.1 Alloxan

Alloxan is a pyrimidine derivative and firstly synthesized in 1838 by Wohler and Liebig. The hyperglycemic property of this chemical was reported many years later by Dunn, Sheehan and Mclethic (1943), where they studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic islets [34]. It has two distinct pathological effects. Firstly, it selectively inhibits glucose induce insulin secretion through its specific inhibition of glucokinase to block glucose phosphorylation and prevent energy release to the islet cells. Secondly, alloxan is able to induce generation of reactive oxygen species through intracellular metabolism of xenobiotic by redox cycling with dialuric acid in a Fenton reaction. These two effects can be assigned to the alloxan because of its specific chemical properties, molecular shape or structure, hydrophilic in nature [35]. Its structure is similar to glucose so that GLUT2 glucose transporter is able to accept and transport alloxan into the cytosol to generate reactive oxygen species in acyclic reaction with the reduction and production of dialuric acid leading to islets cell necrosis and death [35,36]. This toxic action of alloxan is initiated by free radicals formed in this redox reaction with a simultaneous massive increase in cytosolic

calcium concentration leading to a rapid destruction of islet cells. Thus, the pancreatic islets (beta-cells) cell toxicity and the hyperglycemic action of alloxan results to type 1 DM after 72 hours of induction in rats and rabbits [37].

The dose required to induce diabetes depends on the experimental animal species, route of administration, animal nutritional status and researcher experience. A single dose of 150 mg/kg body weight can induce diabetes in a fasting rat but lower dosage may be insufficient in achieving diabetes [38]. Alloxan is effective if properly prepared and administered at an optimum dose and at the expected time because it can be decomposed into non-diabetogenic chemicals while in solution within minutes due to its short half-life [34]. The action of alloxan on the islets cells can be reversed and at the same time the action can be not specific to beta cells. This may result in nephrotoxicity and renal failure [38]. The induced animals need to be maintained on 5% dextrose water for a minimum period of 48 hour post induction to avoid acute hypoglycemic death of animals due to a massive insulin release from the damaged beta cells [39].

2.9.2 Streptozotocin

Streptozotocin is a nitrosourea analogue with lipophilic in nature, antimicrobial property and has also been used as a chemotherapeutic alkylating agent. Streptozotocin is specific to pancreatic beta-cells hence it is used for diabetic study. Streptozotocin is synthesized by *Streptomyces achromogenes* and is used to inhibit insulin secretion by destroying the beta-cells of the islets of Langerhans of the pancreas, a state of insulin dependent DM [40]. It selectively accumulates in pancreatic beta cells via the low affinity to bind with GLUT2 glucose transporter in the plasma membrane, and its toxicity depends upon the DNA alkylating activity of its methylnitrosourea moiety. The methyl group donated from streptozotocin causes DNA damage and fragmentation, leading to diminished cellular NAD^+ and ATP with subsequent beta cells death. Apart from that, protein methylation contributes to the functional defects of the beta cells after exposure to streptozotocin, leading to impaired glucose and insulin homeostasis in both glucose oxidation and oxygen consumption [41]. Streptozotocin can exert its diabetogenic action when administered either intravenously at a dose of 100 mg/kg for 3 days or intraperitoneally at dose of 180 mg/kg for 7 days in mice with 100% success. In addition to the route of administration, other variables such as dose, sex, duration of induction and injection frequency are adapted in order to achieve DM. It is observed that intravenous administration is more effective than intraperitoneal administration in mice [42]. The most effective intravenous dosage to achieve hyperglycemia or diabetes in rats is between 50 mg/kg and 60 mg/kg body weight, but 40mg/kg is less effective and 70 mg/kg is lethal [43]. However, higher doses of 120, 150 and 180 mg/kg are found to be safe and effective in mice [42]. Streptozotocin is costly and has been found to be a suitable model of studying long term diabetes complications as a single diabetogenic dose has been demonstrated to induce DM in most animal species within 24 hours. This method is commonly used as it is effective in producing a good model of Type 1 DM [44].

The advantages and limitations of these methods are summarized Table 1.

Table 1. Summary of advantages and limitations of methods used in diabetes study in experimental animal models

Methods	Advantages	Limitations
Autoimmune islets cells destruction	• New technique • Can be applied for both type 1 and type 2 diabetes mellitus	• Needs expertise, time consuming and not reliable • Rarely used due to its nature
Surgical approach	• Reliable and effective due to reduce islet cell mass • Hyperglycemia achieved within days to weeks	• Needs expertise, costly and requires pre and post operation care with higher motility rate
Genetic animal model	• Provide a new idea in diabetes study • Effective and reliable	• Needs expertise • Expensive and takes time to develop hyperglycemia

Methods	Advantages	Limitations
Steroid therapy	• Simple and affordable • Sometimes effective	• Time consuming • Associated with systemic complications
High sugar high fat diet	• Affordable and less costly • Use in acute study	• Develops postprandial hyperglycemia • Can easily normalize
Hemochromatosis	• Less costly • The damage to pancreas is easily reversible	• Time consuming • Requires higher dose • Can cause liver and pancreatic damages
Spontaneous diabetic model	• Hyperglycemia is achieved at a particular age group • A good model of metabolic syndrome	• Time consuming as breeding is needed • Does not always achieve diabetes
Normoglycaemic rat	• Used to test the diabetogenic activities of a new substance	• Mostly to investigate the substance activity in normal rats • Commonly use as a control group in diabetic research
Alloxan	• Easily available, less expensive and affordable. • May achieve diabetes in 48 hours • No DNA damage.	• not specific, unstable with a very short half life • 5% glucose is required in the first few hours after the induction to avoid post induction hyperglycemia • can cause nephrotoxicity
Streptozotocin	• Reliable and very effective • May achieve diabetes in 24 hours • Can be used for longer experimental study	• Expensive • Can cause DNA damage • Repeated use may cause pancreatic or liver damage • Can cause post induction hypoglycemia

3. CONCLUSION

DM is a life threatening chronic disease with no cure. It has become a problem of great magnitude associated with many medical and social challenges [2]. Emergence of researchers in the field provide many options on how to live with the disease without developing complications but the lasting solution to the problems are not yet discovered [1]. Many of the animal models have allowed experimentation that would be impossible in humans and the two most common beta-cells cytotoxic compounds used in diabetogenic studies in both developed and developing countries are alloxan and streptozotocin [45]. Streptozotocin is predominantly used, it requires a lower dose to achieve the desired hyperglycemia [9] with a higher percentage of success, showing persistent hyperglycemia of plasma glucose [43]. On the other hand, alloxan is mainly used in developing societies despite its short comings [9]. Other methods like pancreatectomy, genetic remodeling and immune mediated beta-cells destructions require more technical skills as well as are associated with higher mortality and miscellaneous expenses [10]. Thus, these methods are rarely used in research but only for academic discussion and serve as an eye opener for new research field. All these methods are observed to cause symptoms of diabetes through the etio-pathogenesis of the disease. It is hoped that this review will guide the researchers in selecting the most appropriate and suitable way to achieve DM in experimental animal model taking into account the advantages and limitations of each technique. It will create opportunities for further research in the relevant field of study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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