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Review

# Animal models and natural products to investigate *in vivo* and *in vitro* antidiabetic activity

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ABSTRACT

Diabetes mellitus is a chronic disease which has high prevalence. The deficiency in insulin production or impaired insulin function is the underlying cause of this disease. Utilization of plant sources as a cure of diabetes has rich evidence in the history. Recently, the traditional medicinal plants have been investigated scientifically to understand the underlying mechanism behind antidiabetic potential. In this regard, a substantial number of *in vivo* and *in vitro* models have been introduced for investigating the bottom-line mechanism of the antidiabetic effect. A good number of methods have been reported to be used successfully to determine antidiabetic effects of plant extracts or isolated compounds. This review encompasses all the possible methods with a list of medicinal plants which may contribute to discovering a novel drug to treat diabetes more efficaciously with the minimum or no side effects.

## 1. Introduction

Recently, over a thousand plants have been reported which may possess antidiabetic potentials [1]. However, so far, relatively few traditional medicinal plants have been scientifically evaluated to prove their safety, potential benefits and effectiveness as antidiabetic agent. Moreover, inadequate data exist for most plants to guarantee their quality, efficacy and safety. This article reviews all the possible aspects of antidiabetic research including the animal models used to investigate antidiabetic activity both *in vivo* and *in vitro* along with the medicinal plants reported for exhibiting antidiabetic activity [2,3].

Animal models are one of the major tools to progress with establishing an effective model to investigate the mechanism of action as well as to explore the efficacy of the active principles and plants claimed to show antidiabetic potentials. Furthermore, the disease itself is heterogenic in nature which leads many ways to cause the diabetes as well as the other conditions related to diabetes. Thus, a single animal model to investigate the efficacy of the drug is not possible owing to the existence of different types of diabetes mellitus [4,5].

In this regard, non-diabetic animals and diabetic animals with impaired glucose tolerance are used as normal control and diabetic control respectively to measure the hypoglycaemic condition. Additionally, tumor necrosis factor-alpha (TNF-$\alpha$) is used to gain insulin resistant diabetic model [6]. It is a matter of great concern that the active ingredients of medicinal plants might not be evaluated effectively in lowering the blood sugar level due to the differences in hepatic metabolism system between human and rodents. The metabolism process happens in several steps, so the active principles have to go through all this pathway and the metabolites are the active agents which reach to the body [7–9].

Sensitivity of the same active principles might vary in different species because of the variations in absorption, distribution, metabolism and elimination (ADME) [2,10–12]. Among all the animal models, the rodents have been widely used in diabetes research for various reasons viz., the time of diabetes induction in the rodents is very short and the maintenance cost is relatively low which cuts down the experiment budget significantly. Furthermore, genetic mutations to induce diabetes have been largely reported in favour of rodents which are significantly greater than any other animal groups [13–15].

## 2. *In vivo* animal experimental models for diabetes mellitus

Diabetes mellitus is induced chemically, surgically or by genetic manipulations (see Table 2). Although, there are some reports that have been shown to use the larger animals to induce diabetes, it is very common to use rodents for the experiments of diabetes [16–18]. Surgical model in large animal has been reported to perform pancreatectomy in dog [19]. In other study, diabetes prone strains have been

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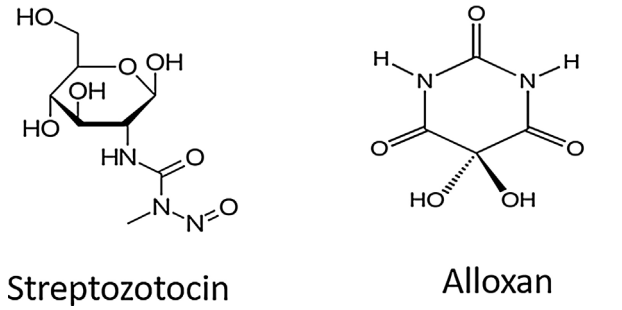


**Fig. 1.** Chemical structures of streptozotocin (STZ) and alloxan (ALX).

introduced to the animals in regard to induce diabetes [20,21].

The classifications of diabetic models can be further narrowed down according to the type of nature of induction of diabetes. The narrowed down animal models are as follows:

1. Spontaneously developed diabetic model (e.g., CBA/Ca mouse, ob/ob mouse, etc.).
2. Experimentally developed diabetic model (e.g., chemically induced by alloxan or streptozotocin; surgically developed by pancreatectomy).

### 2.1. Chemically induced diabetic model

Streptozotocin (STZ) and alloxan (ALX) (Fig. 1) are widely used to induce diabetes mellitus in animals. Both chemicals can be administered through either intravenous (IV), intraperitoneal (IP) or subcutaneous (SC) ways. The mechanisms of action for both chemicals are quite understandable. Both STZ and ALX are selective cytotoxic agents and consequently destroy the pancreatic beta cells selectively. In short, STZ and ALZ are transported to pancreatic beta cells by GULT2 glucose transporter since both are glucose analogues. The STZ splits into glucose and methylnitrosourea. Methylnitrosourea possesses alkylating properties. The alkylation of DNA by methylnitrosourea leads to the destruction of the beta cells. Contrary, the ALX generates reactive oxygen species (ROS) in the presence of glutathione intracellularly. The ROS cause cyclic redox reaction until hydroxyl radicals are produced which eventually lead to the destruction of the beta cells [22–24]. Moreover, some studies have suggested that the complete or partial loss of pancreatic beta cells leads to various complications such as hyperglycemia along with glycosuria, polyuria, polydipsia, hyperphagia, and weight loss [25–27].

The streptozotocin induction causes suppression of insulin secretion by the destruction of pancreatic beta cells. Streptozotocin is a nitrosourea analogue which has a hexose moiety linked to N-methyl-N-nitrosourea moiety. Since the nitrosourea is lipophilic, the cellular uptake of STZ into plasma membrane is fast. Additionally, the STZ is selectively accumulated and transported via GLUT2 glucose transporter [28,29]. Consequently, the insulin producing cells could be STZ resistant if the cell does not express any GULT2 transporter [30–32]. The underlying mechanism of toxic effects of STZ to the pancreatic beta cell is assumed to be taken place by the alkylation of DNA from the interaction of methylnitrosourea moiety of STZ. The consequence of the alkylation initiates a bunch of events which lead to fragmentation of the DNA [33–36] (Fig. 2).

Another hypothesis for STZ mechanism of action claims that the intracellular nitric oxide (NO) donor is responsible for the diabetogenic effects of STZ [37]. Chemically, STZ could liberate NO as it possesses nitroso group. Moreover, effect of NO is attributed to the elevated action of guanyl cyclase and the formation of cGMP by STZ. Therefore, the most toxic alkylating agent known as methyl methanesulphonate is not a NO donor which may suggest that the NO donating could not be considered as the underlying reason for toxic effects of STZ [38].

Additionally, it has been reported to generate ROS along with superoxide and hydroxyl radicals as byproducts of hydrogen peroxide dismutation in hypoxanthine metabolism. A minor effect from the ROS can be expected during the toxic effects in pancreatic beta cells even though it does not play a vital role [39].

On the other hand, the mechanism of action of alloxan includes the reactive oxygen species (ROS). The dismutation of free radicals causes great increase of calcium concentration inside the cell which leads to rapid destruction of the pancreatic beta cells. Dialuric acid (Fig. 3) is a reduction product of alloxan which helps to generate ROS in a cyclic reaction [40–42]. The dose of alloxan needed to induce diabetes is less compared to STZ. Additionally, there is high possibility of death if it is slightly overdosed [43]. Moreover, the tubular cell necrosis in kidney

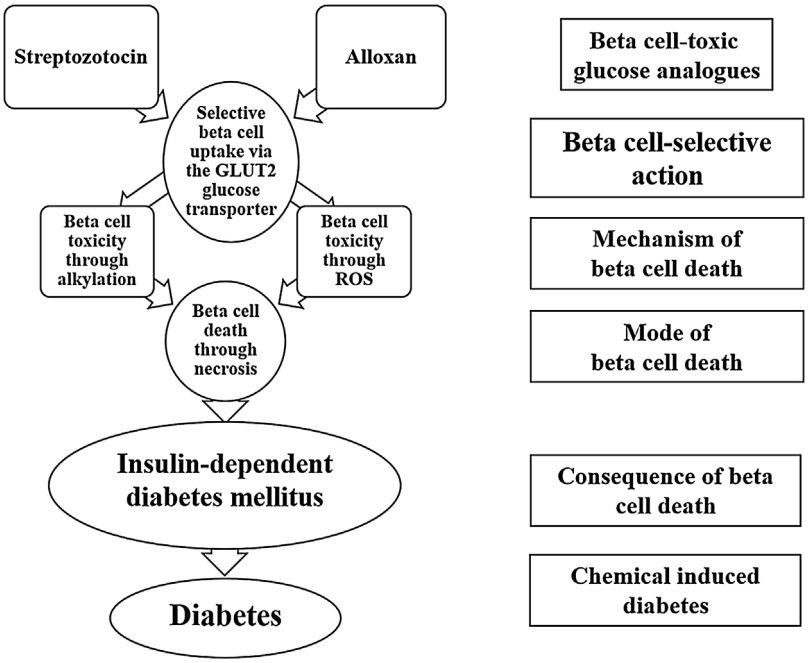


**Fig. 2.** A graphical illustration of the toxic effects of alloxan and streptozotocin in the beta cells.