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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-α) 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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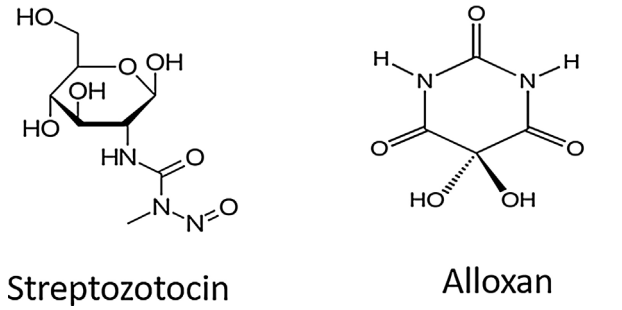

Fig. 1. Chemical structures of streptozotocin (STZ) and alloxan (ALX).

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

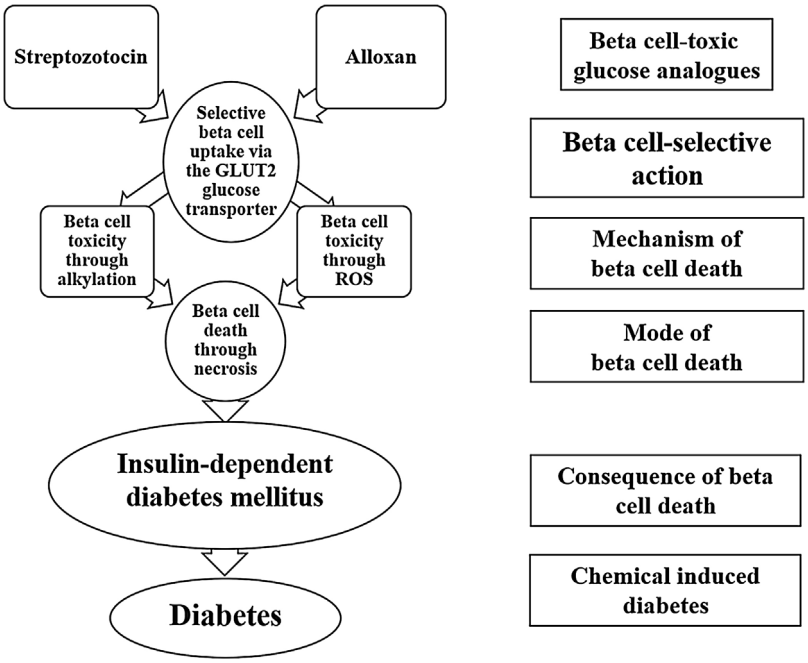

Fig. 2. A graphical illustration of the toxic effects of alloxan and streptozotocin in the beta cells.

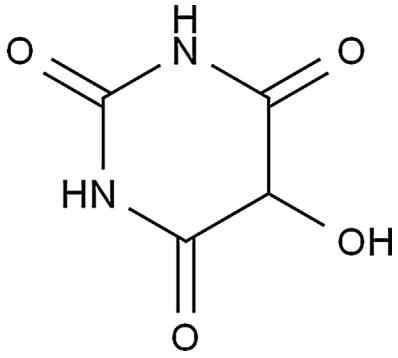

Fig. 3. Structure of dialuric acid i.e., reduction product of alloxan responsible for ROS generation.

has been reported to be observed due to high dose of ALX induction [22]. Since, the correct dosage regime for the induction of diabetes in animals is very important matter for both the STZ and ALZ, scientists have reported the doses extensively in several scientific communications.

The chemical profile, mechanisms of action and doses of streptozotocin and alloxan in various diabetic animal models are presented in Table 3.

2.2. Surgically induction of diabetes

Apart from the chemical induction, the surgical removal of pancreas is an alternative in regard to reduce toxic side effects of chemically induced diabetes. It has been reported to develop diabetic animal model of some species viz., rats, dogs, pigs, etc. [44,4]. However, the limitations of this technique have also been reported by many published studies. The highly complex nature of the technique demands great expertise to perform the surgery. Additionally, the cost of the surgery room set up, large amount of analgesic and antibiotic, post-surgery pancreatic enzyme supplement and risk of animal infection are major drawback of this technique. Moreover, there have been many attempts to perform partial pancreatic surgery which is yet to be developed or acclaimed to achieve desired diabetogenic action [45,46,10,47].

2.3. Spontaneous and transgenic animal models

Several diabetic animal models have so far been developed and reported where no chemical or surgical methods have been used to induce diabetes. In these instances, diabetes is developed either spontaneously or by genetic alteration. Animal models have been listed in Table 1. In some cases, the insulin resistant has been reported as comorbid to obesity, dyslipidemia, and hypertension which helps to investigate the diabetes complications similar to type II diabetes of human being [48,13,8,5].

There are many methods available in these criteria. Each of the models has different underlying mechanism. For instance, db/db mice develops hyperglycemia rapidly, however, Goto–Kakizaki rat which is genetically lean type II diabetic model develops hyperglycemia spontaneously in comparison to glucose-intolerant nondiabetic Wistar rats. BB rats can develop diabetes by 12 weeks whereas NOD mouse usually takes 12–30 weeks to develop hyperglycemia [46,49,50].

2.4. Virus-induced diabetic model

It has been reported that the juvenile diabetes (Type I) has a link with virus infection as well as an autoimmune condition on specific beta cell [51]. A virus named encephalomyocarditis which is a D-variant has been reported to destroy the pancreatic beta cells [52,53]. Two of the virus susceptible animals are male ICR Swiss (adult) mice and male C3H/HeJ (adult) mice whereas the former was found more prone to the induction of diabetes than the later. The experiment was further confirmed by the pre-treatment of a potent immunosuppressant named cyclosporin. The consequences of the induction of immunosuppressant

Table 1
Classification of diabetic models in various animals.

Model category	Obese	Non-obese
(I) Spontaneous or genetically derived diabetic animals	1. CBA/Ca mouse, 2. ob/ob mouse, 3. db/db mouse, 4. KK mouse, 5. KK/Ay mouse, 6. NZO mouse, 7. NONcNZO10 mouse, 8. TSOD mouse, 9. M16 mouse, 10. BHE rat, 11. Zucker fatty rat, 12. WDF/Ta-fa rat (Winster fatty rat), 13. ZDF rat, 14. obese SHR rats, 15. SHR/N-cp rat, 16. JCR/LA-cp rat, 17. OLETF rat, 18. Obese rhesus monkey	1. NOD mouse (Non obese diabetic mouse), 2. ALS/Lt mouse, 3. (Akita) mutant mouse, 4. GK rat, 5. Cohen diabetic rat, 6. C57BL/6 Torri rat (Non-obese), 7. BB rat (Bio Breeding), 8. WBN/Kob rat, 9. eSS-rat
(II) Diet/nutrition induced diabetic animals	1. C57/BL 6J mouse, 2. Spiny mouse, 3. Sand rat	–
(III) Chemically induced diabetic animals	GTG-treated obese mice	1. Neonatal STZ rat, 2. Low-dose ALX or STZ adult rats and mic
(IV) Transgenic/knock-out diabetic animals	1. β-3 receptor knockout mouse, 2. Uncoupling protein (UCP1) knockout mouse	1. IRS-1, IRS-2, GLUT-4, PTP-1B, and others PPAR-g tissue-specific knockout mouse, 2. Glucokinase or GLUT 2 knockout mice, 3. Human islet amyloid polypeptide (HIP) over expressed rat, 4. RIP-LCMV transgenic mice, 5. Murine Hsp60 transgenic NOD mice
(V) Virus induced diabetic animals	–	1. Male ICR Swiss mice (susceptible to D-variant of encephalomyocarditisvirus (EMC-D), 2. adult C3H/HeJ male mice (Resistant to EMC-D), 3. DBA/2 mice (Susceptible to NDK25, a variant of encephalomyocarditis virus which has been cloned from the M variant of encephalomyocarditis virus)

ALS: alloxan sensitive; ALX: alloxan; BB: Bio Breeding; C3H/HeJ: lipopolysaccharide response, locus; DBA: Dilute Brown Non-Agouti; GLUT: glucose transporter; GK: Goto-Kakizaki; GTG: gold thioglucose; ICR: impaired cytokine response; IRS: insulin receptor substrate; JCR: James C Russel; KK: Kuo Kondo; KK/Ay: yellow KK obese; NZO: New Zealand obese; OLETF: Otuska Long Evans Tokushima fatty; PPAR: peroxisome proliferator activated receptor; PTP: phosphotyrosine phosphatase; SHR/N-cp: spontaneously hypertensive rat/NIH-corpulent; STZ: streptozotocin; TSOD: Tsumara Suzuki obese diabetes; VMH: ventromedial hypothalamus; ZDF: Zucker diabetic fatty.
After [1].

Table 2
Advantages and disadvantages of various classes of diabetic animal models.

Model category	Advantages	Disadvantages
(I) Spontaneous diabetic animals	1. The spontaneous origin diabetes has many varieties which depicts more precisely the complex nature of diabetes in human being [48,13,8,5]. 2. Many animal methods available according to onset of action or mechanism of action. Some models are very rapid to develop diabetes whereas some models depict variety mechanism of actions including insulin resistant of type II diabetes [46,49,50].	1. Apart from showing different mechanism of action, the animal models are highly monogenic when human ADME system is more complex than these models [2,10–12]. 2. The animal model is not very available and post-diabetes maintenance is a major challenge to keep the animals healthy [46,49,50].
(II) Diet/nutrition induced diabetics	1. The experiment is cost effective since diabetes is induced by diet or nutrition which reduces cost of experiment. 2. Safer than chemicals and less toxicity to vital organs.	1. Long period is needed since the animals are fed over diet to increase blood sugar level.
(III) Chemical induced diabetic animals (STZ or ALX)	1. The toxicity is very selective to pancreatic beta cells which avoids other complications. 2. Mortality rate is relatively low since the correct doses of both STZ and ALX are earlier reported [3,23].	1. The treatment is less stable and reversible as the rate of beta cell generation is high. 2. Toxicity in other vital organs is also a major concern.
(IV) Surgical diabetic animals	1. The model is free from toxicity of other vital organs [44,4]. 2. The model is developed rapidly and it can be developed very selectively by only removing the pancreatic beta cells [44,4].	1. It is very expensive to set up the whole operation. 2. It demands high level of technical expertise to perform the surgery [45,46,10,47]. 3. The postoperative antibiotic and hormone supplements are very costly and requires high level of monitoring [45,46,10,47].
(V) Transgenic/knock out diabetic animals	1. The model is very selective and able to shed lights on a single gene extensively.	1. The model is very complex and expensive to develop.

was found to increase in diabetic conditions in both cases [54].

3. *In vitro* animal models

In early studies, insulin-like activity had been measured by the glucose uptake into the fat cells isolated from epididymis of the animals. Adipose tissue of the rat epididymal fat pad had been widely used in this study. The epididymal rat adipose tissue was incubated in glucose containing media and the glucose uptake was measured by the glucose concentration of the media or the oxygen consumption in Warburg vessels. Glucose uptake by incubated rat epididymal adipose tissue was predominantly regulated by the rate of glucose transport across the cell membrane. Another type of experiment was done with the radio labelled glucose where $^{14}CO_2$ was captured and counted. The $^{14}CO_2$ produced from the Iodine labelled ^{14}C glucose was measured in this method [55,56].For the small amount of insulin quantification, a manometric assay was used to calculate the total gas exchange [57,58]. Later on, some modification in this method was done in various studies. Among them, the isolated fat cells, 3T3-L1 adipocytes and primary cultured adipocytes were found to be very effective in testing insulin-like activity through *in vitro* models [59].

4. Recommendations of animal models for evaluating antidiabetic activities

Both *in vivo* and *in vitro* models have their own advantages over other models. For antidiabetic research, the *in vitro* models are more cell specific and less time consuming. The effects could be easily analysed by various assays. In contrast to that, the *in vivo* methods are more time consuming and costly. Additionally, it needs a lot of expertise to

Table 3
Comparison of streptozotocin and alloxan.

	Streptozotocin	Alloxan
Chemical name	2-Deoxy-2-([(methylnitrosoamino)carbonyl]amino) D-glucopyranose	2,4,5,6-Tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone
Chemical characterization	Cytotoxic methylnitrosourea moiety (Nmethyl-N-nitrosourea) attached to the glucose (2-deoxyglucose) molecule; glucosamine derivative	Oxygenated pyrimidine derivative; barbituric acid derivative (5ketobarbituric acid)
Chemical properties	1. Hydrophilic, beta cell-toxic glucose analogue 2. Relatively stable at pH 7.4 and 37 °C (at least for up to 1 h)	1. Very hydrophilic, beta cell-toxic glucose analogue (partition coefficient −1.8); weak acid 2. Chemically unstable (half-life of 1.5 min at pH 7.4 and 37 °C,decomposing to alloxanic acid); stable at acid pH
Chemical reactivities	1. DNA alkylating agent 2. Protein alkylating agent 3. NO donor	1. Thiol reagent that is reduced to dialuric acid in the presence of GSH and other thiols 2. A protoxin; intracellular metabolism of this xenobiotic generates toxic ROS through redox cycling with dialuric acid over a long period of time (> 1 h) 3. Compound 305, a non-toxic alloxan-GSH adduct of unknown structure with a characteristic absorbance at a wavelength of 305 nm; a small amount is formed during each redox cycle
Mode of toxicity	DNA alkylation	Generation of ROS
Dose (mg/kg)	Rat: 35–65 (i.v./i.p.) Mice: 100–200 (i.v./i.p.) Hamster: 50 (i.p.) Dog: 20–30 (i.v.) Pig: 100–150 (i.v.) Primates: 50–150 (i.v.)	Rat: 40–200 (i.v./i.p.) Mice: 50–200 (i.v./i.p.) Rabbit: 100–150 (i.v.) Dog: 50–75 (i.v.)

i.v.: intravenous; i.p.: intraperitoneal.

Table 4
Medicinal plants with antidiabetic effects.

No	Plant name	Family	Type of extract	Part used	Activity and references
1	*Alangium lamarckii*	*Alangiaceae*	Alcoholic	Leaf	Antidiabetic [84]
2	*Albizia odoratissima*	*Mimosaceae*	Methanol	Bark	Antidiabetic [85]
3	*Axonopus compressus*	*Poaceae*	Methanol	Leaf	Antidiabetic [86]
4	*Berberis vulgaris*	*Berberidaceae*	Aqueous	Root	Hypoglycaemic [87]
5	*Brassssica juncea*	*Cruciferae*	Aqueous	Seed	Hypoglycemic [88]
6	*Caesalpinia digyna*	*Fabaceae*	Methanol	Root	Antidiabetic [89]
7	*Catharanthus roseus*	*Apocynaceae*	Methanol	Leaf	Hypoglycemic [90]
8	*Centaurium erythrea*	*Gentianaceae*	Aqueous	Leaf	Antidiabetic [91]
9	*Chaenomeles sinensis*	*Rosaceae*	Ethyl acetate	Friut	Antidiabetic [92]
10	*Cocos nucifera*	*Arecaceae*	Hydro-methanol	Leaf	Antihyperglycaemic [93]
11	*Costus speciosus*	*Costaceae*	Hexane	Rhizome	Antidiabetic [94]
12	*Cyclocarya paliurus*	*Cyclocaryaceae*	Aqueous, petroleum ether, chloroform, ethyl acetate &n-butanol	Bark	Hypoglycaemic [95]
13	*Dillenia indica*	*Dilleniaceae*	Methanol	Leaf	Antidiabetic [96]
14	*Embelia ribes*	*Myrsinaceae*	Hexane	Berries	Antidiabetic [97]
15	*Hybanthus enneaspermus*	*Violaceae*	Alcoholic	Whole plant	Antidiabetic [98]
16	*Lippa nodiflora*	*Verbenaceae*	Methanol	Whole plant	Antidiabetic and hypolipidemic [99]
17	*Lithocarpus polystachyus*	*Fagaceae*	Ethanol &Aqueous	Leaf	Hypoglycaemic [100]
18	*Marrubium vulgare*	*Lamiaceae*	Methanol	Aerial parts	Hyperglycaemic and dyslipidemic [101]
19	*Ocimum sanctum*	*Lamiaceae*	Hydroalcoholic	Aerial parts	Antidiabetic [102]
20	*Opuntia streptacantha*	*Cactaceae*	Ethanol	Leaf	Antihyperglycaemic [103]
21	*Psidium guajava*	*Myrtaceae*	Ethanol	Fruit	Antihyperglycaemic [104]
22	*Semecarpus anacardium*	*Anacardiaceae*	Milk	Nut	Antidiabetic [105]
23	*Prosopis glandulosa*	*Fabaceae*	Gelatine/Jelly	Whole plant	Antidiabetic [106]
24	*Ophiopogon japonicus*	*Asparagaceae*	Ethanol	Root	Hypoglycaemic [107]
26	*Setaria italica*	*Poaceae*	Aqueous	Seed	Antihyperglycaemic [108]
25	*Solanum torvum*	*Solanaceae*	Methanol	Fruit	Antihyperglycaemic [109]
26	*Cassia auriculata*	*Caesalpiniacae*	Aqueous	Leaf	Antihyperglycaemic [110]
27	*Zygophyllum album*	*Zygophyllaceae*	Ethanol	Whole plant	Antidiabetic [111]
28	*Vitex negundo*	*Lamiaceae*	Methanol	Leaf	Antihyperglycaemic [112]
29	*Viscum schimperi*	*Viscaceae*	Methanol	Aerial parts	Antihyperglycemic &Hypolipidaemic [113]
30	*Symplocos cochinchinensis*	*Symplocaceae*	Hexane	Leaf	Antidiabetic [114]
31	*Enicostemma littorale*	*Gentianaceae*	Aqueous	Whole plant	Antidiabetic [115]
32	*Vaccinium arctostaphylos*	*Ericaceae*	Ethanol	Fruit	antidiabetic [116]
33	*Solanum xanthocarpum*	*Solanaceae*	Aqueous and methanol	Leaf	Antihyperglycaemic [117]

effectively perform the *in vivo* experiments to obtain reproducible data. However, the human body system is a complex system which cannot be revealed by *in vitro* assays only. For that reason, the efficacy of the molecules of samples should also be tested through *in vivo* system for better understanding. In conclusion, the *in vitro* animal models could specially be recommended for mechanism specific research and also primary stage research to find out the exact target molecule or receptor. However, the *in vivo* models are recommended for the further studies to develop a drug as well as to evaluate toxicological profiles.

5. Medicinal plants with antidiabetic effects

Medicinal plants continue to be an important therapeutic support for alleviating disorders of humankind. Following is the list of some important traditional medicinal plants that have recently been scientifically proven for their antidiabetic potential. These plants may further provide lead for the discovery of new plant based antidiabetic agents with safe profiles (Table 4).

6. Role of flavonoids as an antidiabetic agent

Flavonoids are considered as one of the most abundant secondary metabolites of the plant. Approximately, more than 8000 unique flavonoids from different plants have so far been isolated and characterized through various isolation and spectroscopic techniques [60]. In addition to that, the flavonoids are mostly found in fruits, vegetables, nuts, seeds, stem, flowers, etc. [61]. The flavonoids are low molecular weight compounds and have been reported to exert various important pharmacological effects including the cell synthesis [62–65]. Moreover, the scientific claims for flavonoids as a cure of diabetes is noteworthy owing to its wide range of mechanisms of action [66].

Briefly, diabetes mellitus has been stated to hinder the protein, carbohydrate and fat metabolism which result in hyperglycaemia [67]. The first line of therapeutic measurement towards DM is to keep the blood glucose level normal and attain glucose homeostasis. The attempt to achieve glucose homeostasis is quite successful at present but the therapeutic agents are not free from side effects and in some cases, have been proven extremely toxic with high mortality. In this circumstance, the worlds scientists are looking for a better alternative to cure DM [68]. The flavonoids are yet to be commercialized; however, they have gained the prime attention of healthcare professionals for their potentials in the ailment of diabetes [69]. The presence of flavonoids has been confirmed vigorously in the plants which have potential antidiabetic effects. However, a plant contains thousands of compounds and most of the compounds are yet to be isolated and characterized. In this regard, more sincere research efforts must be orchestrated to discover more efficacious antidiabetic agents to help mankind.

Recently, various experimental techniques have been usually used to evaluate the antidiabetic activity of traditional medicinal plants containing flavonoids as one of the major phytoconstituents. Firstly, the experiments are carried out with the crude extract of the medicinal plant to confirm the bioactivity and then subsequently with the isolated flavonoids to confirm active principles responsible for the antidiabetic effect of the particular traditional medicinal plant. Table 5 describes about the research studies in which flavonoids were found as an active principles responsible for the aforementioned effect.

7. Therapeutic approach towards diabetes

Very often the therapeutic approach of diabetes is quite costly and most of the times they are not free from various deleterious effects. The most common and severe side effect associated with antidiabetic agents

Table 5
Some therapeutic approaches in animal model to treat diabetes mellitus by the isolated flavonoids.

Therapeutic approach	Flavonoids	Result
1. *Averrhoa carambola* isolated flavonoid on glycogen synthesis and insulin secretion	Apigenin-6-C-l-fucopyranoside	Increased insulin secretion and potentiated glucose-induced insulin secretion in hyperglycemic rats [118]
2. *Acacia auriculiformis* poly flavonoids on antidiabetic factor	Gallic acid, caffeic acid, catechin, rutin, quercetin, myricetin and kaempferol	Significant suppression on α-amylase and -glucosidase [119]
3. Effect of *Bauhinia forficata* on serum glucose	Kaempferol-3,7-O-(α)-rhamnoside	Hypoglycemic effect was observed in alloxan induced diabetic rats [120]
4. Icariin in diabetic rats on mitochondrial oxidative stress	Icariin	Improvement in overall complication of diabetes in steptozotocin induced rats [121]
5. Kaempferitrin on glucose uptake on skeletal muscles	Kaempferitrin	Insulin-mimetic role of kaempferitrin in glucose homeostasis [122]
6. Isolates from *Euonymus alatus* in glucose uptake	Kaempferol and quercetin	Potential ameliorated hyperglycemia observed, due to glucose uptake in mature 3T3-L1 adipocytes [123]
7. An isolate exposure on chronic hyperglycemic-cell	Kaempferol	Restoration of high glucose-attenuated intracellular cAMP and ATP production [124]
8. Flavonoid isolated as insulinomimetic on glycemia and glucose uptake	Kaempferitrin	Lowering blood glucose level and ^{14}C uptake of glucose in peripheral muscles of rat [125]
9. Inhibition of intestinal and renal Na-glucose cotransporter	Naringenin	Inhibition of intestinal glucose uptake and renal glucose reabsorption was observed [126]
10. Combined effect of flavonoid in type 2 diabetic rat	Naringin and hesperidin	Synergistic insulin releasing effect [127]
11. Antidiabetic activity of isolated in normoglycemic rats	Naringin	Diminishing the carbohydrate absorption from intestine and reduced post prandial glucose levels [128]
12. Antidiabetic activity of isolated flavonoids from *Tetracera indica* Merr.	Wogonin, norwogoin and techtochrysin	Enhancement of insulin like activity and insulin sensitizing activity in 3T3-L1 preadipocyte and adipocyte [129]

is hypoglycaemia. Gaining weight is also considered as severe effect particularly associated with sulfonylurea therapy due to excessive insulin release [70]. The risk of developing excessive hypoglycaemic condition is more associated with the use of sitagliptin or linagliptin in combination with sulfonylurea or insulin therapy. Other side effects can also occur due to DPP-4 inhibitors (dipeptidyl peptidase – IV inhibitors) such as – headache, nausea, nasopharyngitis and hypersensitivity or skin reaction [71]. With alpha-glucosidase inhibitors treatment mild symptoms may also occur such as flatulence and diarrhoea. Metformin might cause some mild adverse effects like mild anorexia, nausea, diarrhoea, abdominal discomfort and mostly are gastrointestinal. The existing anti-diabetic drugs have both mild and severe adverse effects like hypoglycaemia, weight gain, gastrointestinal effects, headache, nausea, abdominal discomfort and so on with prolonged use [72]. Thus, it is urgent need to find out more safe and effective classes of anti-diabetic medications to counteract the aforesaid problems associated with current regimens [73]. Furthermore, diabetes is comorbid in nature which makes the treatment more expensive. Consequently, financially well-off people are getting more interested in alternative medicines (e.g., herbal medicines) [74]. Medicine prepared from natural plant is considered to be free of side effects and also less toxic compared to the synthetic medicine [75]. With respect to treat or manage diabetes, several traditional plants have been effectively used by people across the world for centuries. More than 1200 plant species are utilized to treat diabetes, however, a very limited number of them have been reported to exhibit good hypoglycaemic effect while evaluating their antidiabetic properties through experimental animal models and in clinical experiments using human subjects [1]. The medicinal plant species serve as beneficial outcome of oral hypoglycaemic agents to develop new pharmaceutical leads and also provide important sources to existing therapies as a dietary supplement [70].

8. Insulin-like and insulin-sensitizing activity assay

This study is designed to evaluate the insulin-like activity and insulin-sensitizing activity of the extract, fractions, and isolated compounds. Some of the currently available techniques are given in Table 6. In short, the hypoglycemic activity is determined by blood sugar determination of the rodent [76] and also by hypoglycaemic seizures in mice according to European Pharmacopoeia, 3rd edition 1997. In other study, cells and tissues are used to evaluate insulin-like activity by several methods, epididymal fat pad [77], fat cells isolated by digestion with collagenase [78], 3T3-L1 adipocytes [79], glucose transport assays [80], etc.

Table 6
Assay of insulin and insulin-like activity.

Bioactivity	Methods
Hypoglycemic effects	1. Blood sugar determinations in rodent [76,130], 2. Hypoglycemic seizures in mice [131]
Insulin target cells/tissues	1. Epididymal fat pad [77], 2. Fat cells isolated by digestion with collagenase [78], 3. 3T3-L1 adipocytes [79], 4. Assays of glucose transport, (a) Total glucose uptake [132], (b) Transport of 2-deoxy-glucose [80], (c) 2-NBDG uptake [129] 5. Adipocyte plasma [133]

Various research studies have strongly emphasised that there is a need to discover the better alternative of insulin to reduce the treatment cost as well as safety reasons. The natural products are considered as the potential source of that alternative, but the better alternative is yet to be discovered. In this regard, all these aforementioned animal models (Table 1) have been effectively used to investigate the potential bioactivity of the natural or synthetic products in the absence of insulin [2,10,12].

9. Prospect of medicinal plants as an antidiabetic agent

Since the disease itself is complex so a single animal model is not enough to investigate the underlying cause of diabetes. At the same time, the traditionally used plants cannot be successfully introduced to the market without a thorough investigation of all possible mechanisms reported [3,81,4].

A recent study carried out by Tufts University Centre shows that developing a novel therapeutic drug costs $2.6 billion and 12–14 years on an average till it receives the Food and Drug administrations (FDA) approval which is 145% increase from 2003. However, to make the medicinal plants commercially available for people is the ultimate goal of natural product research. Therefore, a very comprehensive study is

essential to reach the goal. A thorough literature review of all reported antidiabetic plants is the first stepping stone for natural product research. The plant list should be narrowed down according to certain criteria viz., traditional uses, hypoglycaemic activity proved experimentally, evidence of less toxicity reported scientifically and abundance of plant naturally [82,83].

10. Conclusion

The review has documented a wide range of animal models and medicinal plants used for investigating the antidiabetic activity. The diabetes mellitus is a complex disorder and the human physiology is very complex. Hence, the aim of this literature-based study was to review the existing animal models in the literature along with the potential of medicinal plants with respect to discovering a new alternative to existing drugs. Moreover, the paper also states the drawbacks of utilizing certain animal models and focuses on the specific animal model for suitable investigation. However, the paper suggests more in-depth review on natural product research for the ailment of diabetes that is needed. Finally, the review draws attention of the researchers that a proper documentation of all reported antidiabetic medicinal plants is still warranted in regard to finding out more efficacious antidiabetic agents.

Conflict of interest

The authors have declared that there is no conflict of interest.

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