A review on comparative outcome of chemical induced hyperglycaemia

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ABSTRACT

With the advancement of days, patients with diabetes become household. Many synthetic, semisynthetic, and pharmacological agents have been introduced but fail to neutralize. Hence, this review aims to outline diabetic inducing agent and their pharmacology for new drug screening. Different search engines such as google scholar, PubMed, ScienceDirect, and Elsevier were used to do this review. Our study reports the pharmacology of diabetic inducing agents alloxan monohydrate and streptozotocin. This review will help researchers to choose the right chemicals based on methodology.

Keywords: Alloxan, chemical-induced diabetes, hyperglycemia, streptozotocin,

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterizing high blood sugar levels with abnormalities in the metabolism of carbohydrates, proteins, and fats due to defective insulin secretion or actions [1]**.** Diabetes is a severe health issue affecting more than 62 million people per year in India. This figure is expected to raise 79.4 million by 2030[2]. Moreover, it is assumed that over 439 million individuals will be affected by diabetes by the year 2030 [3].

Diabetes mellitus can be divided into 'Insulin-dependent DM' (IDDM), 'Type-1 DM' and 'Non-insulin-dependent DM' (NIDDM), or 'Type-2 DM'. Type-1 DM is also called juvenile diabetes because it is predominant in juveniles compared to adults. In this type, β cells of pancreatic islets are destructed by autoimmune mechanisms and insulin deficiency and can be managed by administering insulin from outside. On the other hand, type-2 DM is more common in adults due to peripheral insulin resistance [4].

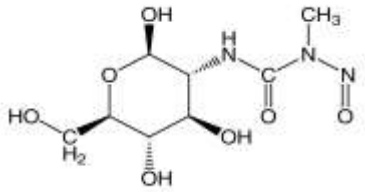
Over the years, researchers have constantly been looking for an effective solution for the prophylaxis and treatment of diabetes. For the screening of the antidiabetic agents, many experimental models have been developed that provide insight into the disorder's

pathology. There are several ways to induce diabetes, such as treating with chemical agents, surgical procedures, or manipulating the genetics of the experimental animals. Streptozotocin (STZ) and Alloxan (ALXN) are widely employed among usually used chemical agents for the induction of diabetes [5].

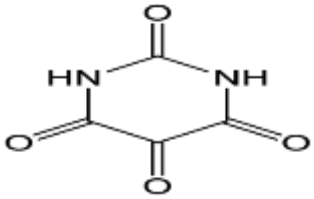
COMPARISON BETWEEN DIABETOGENIC AGENTS

Chemistry of STZ and ALXN

Chemically STZ is named as 2-deoxy-2-{3-(methyl-3-nitrosoureido)-D-glucopyranose} synthesized by *Streptomycetes achromogene.* It is a hydrophilic glucose analog. ALXN is a very hydrophilic, weak acid glucose analog [6]. It is chemically (2,4,5,6 tetraoxypyrimidine or 5,6-dioxyuracil) and synthesized by the oxidation of uric acid [7]**.** STZ is more stable than ALXN at 37˚C and pH 7.4, having a half-life of 1.5 minutes and up to 1 hour, respectively.



Structure of streptozotocin



Structure of Alloxan

Mechanism

Previous studies have shown that both STZ and ALXN break DNA strands and stimulate nuclear poly (ADP-ribose) synthetase, causing a reduction of intracellular NAD level and inhibiting the synthesis of proinsulin. ALXN-induced DNA breakage and proinsulin synthesis inhibition can be protected by superoxide dismutase (SOD), Catalase (CAT), and scavengers of oxygen radicals. However, scavengers of radicals cannot perform the same in the case of STZ. The nuclear poly (ADP-ribose) inhibitors inhibit proinsulin synthesis but cannot protect against the breakage of DNA strands [7].

In vitro study of the effect of STZ and ALXN exhibited the involvement of STZ but not ALXN in the DNA damage process in isolated islet cells of mice. The effects of these two agents on DNA repair synthesis were compared using the autoradiographic method. Dimethyl urea (hydroxyl radical scavenger) and poly (ADP-ribose) inhibitors synthetase enzyme, theophylline, and nicotinamide protected DNA repair activity induced by STZ treatment. Therefore it can be said that STZ affects the β cells by DNA damage mechanism [5].

The mechanism of inducing diabetes by STZ and ALXN is now well established. These two agents enter the pancreas via glucose transporter GLUT2 and accumulate in the β cells. The reactive oxygen free radicals are mainly responsible for causing β cell damage in the pancreas, causing a diabetic state. However, the generation of the radicals by STZ and ALXN is different in origin. First, Dialuric acid, the reduction product of the ALXN, is generated by a cyclic redox reaction catalyzed by intracellular thiols such as

glutathione. Then dialuric acid is autoxidized to produce hydrogen peroxide, superoxide radicals, and hydroxyl radicals in a reaction catalyzed by iron (Fenton Reaction). Hydroxyl radicals cause an increase in cellular Ca^{2+} levels. Since β cells have low antioxidative properties, finally, β cells die due to hydroxyl radical's oxidative stress and produce insulin-independent diabetes. ALXN also inhibits glucokinase's action, inhibiting the secretion of insulin induced by high blood glucose levels. On the other hand, STZ causes DNA damage by the alkylation process, which triggers poly ADP ribosylation leading to NAD^+ and ATP depletion in the cells. Increased dephosphorylation of ATP forms superoxide radicals by supplying a substrate for the enzyme xanthine oxidase. Hydroxyl radicals, as well as hydrogen peroxides, are also formed at the end of the reaction. After accumulation, streptozotocin is converted into two moieties, namely methyl nitrosourea and glucose. Methylnitrosourea produces insulin-dependent diabetes by modifying biological macromolecules, DNA, and β cells. Moreover, STZ causes DNA damage by liberating excess nitric oxides (NO). These actions finally cause necrosis of β cells and produce a diabetic-like state.

Routes of administration and dose

Factors such as administration of routes, species, and nutrition determine the effect of ALXN on the induction of hyperglycemia. Rats or mice can be made diabetic by administering ALXN by parenteral routes like intravenous (IV), intraperitoneal (IP), and subcutaneous (SC)**.** A dose of 65 mg/kg body weight (BW) is preferable in rats for intravenous administration. For induction of diabetes, two to three times higher doses are required by IP or SC routes than the IV route. Rats usually need more than a dose of 150 mg/kg BW by IP routes for ALXN-induced diabetes**.** A high level of blood glucose has a protective action against ALXN action. It is also observed that ALXN can induce diabetes more easily in fasted experimental animals [8].

The most preferable routes of administration for STZ are IV and IP. The usual dose of 40-60 mg/kg BW is used in adult rats by single dose IV administration. IV administration of STZ in a single dose of 40-60 mg/kg BW but not less than 40 mg/kg BW can induce diabetes. STZ can also induce diabetes (IDDM), especially in mice, by activating immune mechanisms when administered in multiple low doses. STZ at the dose of 100 mg/kg BW can induce diabetes (NIDDM) in rats if given on the day of birth by IV or IP route. After 1 month of administration of a single dose of STZ (30, 35, 40, and 50 mg/kg, i.p.), there was a high incidence of permanent and optimum type-1 DM with a lower mortality rate in mice. In the same study, mice were administered a single dose of ALXN by SC (120, 110, 100, 90, and 80 mg/kg) and IP (140, 120, 100, and 80 mg/kg) routes. After 30 days, ALXN at the dose of 40 mg/kg and 120 mg/kg showed optimal induction of diabetes with lower mortality rates by IP and SC routes, respectively. ALXN in a single high dose (200 mg/kg, i.p.) induced Type-1 DM in Sprague-Dawley rats with an incidence and mortality rate of 70% and 10%, respectively. Rats were considered Type-1 diabetic if they had shown a high level of ketone in blood after ALXN treatment. A high dose of intravenous treatment was lethal.

Moreover, animals with higher mortality rates were observed in the multiple-dose treatment of ALXN. One can manage intense hypoglycemia associated with the first 2 days of ALXN treatment by providing carbohydrates and fluids. Long-acting insulin is preferred over rapid-acting insulin to manage hyperglycemia induced by ALXN treatment [9].

Investigations of pathophysiology and complications in diabetes using STZ and ALXN models

STZ (50 mg/kg) reduces intermeal intervals at 4-9 days after intravenous injection. This may be due to hyperphagia, which is developed at 4-6 days of STZ treatment and seen to increase over 2 weeks. However, rats show glucosuria and polydipsia on the first day but cannot produce hyperphagia in the first 3 days after STZ administration. The effect of STZ (60 mg/kg, IP) on fluid and electrolytes balance in rats was observed daily, before and 21 days after the drug treatment. An increase in water intake and urine output was seen on the first and next day of treatment. On the first 3 days, there was a decline in food intake, but gradually increased intake was observed after that. On the last day, a 60% increase in the food intake was observed in the STZ treated group compared to control animals. Though increased excretion of Na^+ was seen after the STZ administration, at the end of the study, intake and output came to a similar level. However, the excretion of K^+ in urine was reduced. Therefore, a reduced concentration of plasma Na^+ but not K^+ was observed [4].

In vitro investigation in isolated hypothalamic cells of adult diabetic rats exhibited reduced release of somatostatin (inhibitor of growth hormone). The rats were diabetic by administering STZ (65 mg/kg, IP) and ALXN (200 mg/kg, SC). The release of somatostatin from hypothalamic cells of obese diabetic C57 BL/Ks dB/db mice was similar to that of the usual littermates. However, rats starved for 5 days showed an insignificant reduction of somatostatin release. After STZ (40 mg/kg) injection in multiple small doses, hyperglycemia was induced, associated with insulitis and type C viruses in the β cells of mice within 5-6 days. STZ in multiple subdiabetogenic treatments in rats and ALXN in multiple injections in mice induced hyperglycemia in higher doses but not insulitis. The STZ-induced diabetes was partially protected by Rabbit antimouse lymphocyte serum (ALS) (0.5 ml/mouse, i.p.) but with 3-O-methyl-D-glucose (3-OMG) (0.22 mmol/mouse, IP) or nicotinamide (0.22 mmol/mouse, IP) significantly protected the diabetogenic effect. When an intravenous infusion of alloxan (50 mg/kg) and streptozotocin (30 mg/kg) was administered in adult beagle dogs, there was gradual destruction of β cells but not α and δ cells of islets of Langerhans. After 2 hours of infusion, nuclear chromatin clumping appeared, vacuoles in mitochondria, dilated endoplasmic reticulum, and swollen secretory granules. After 10 hours, β cells showed intense degradation, and the plasma membrane was disrupted within 24 hours with the coalescence of adjacent β cells. Dogs started showing hyperglycemia within 2 hours with severely increased glucose levels within 6-14 hours, which became permanent by 24 hours. At 6-10 hours, plasma insulin showed a sharp increase that returned to normal 24 hours. Plasma glucagon level showed reciprocal change with insulin exhibiting marked increase a 10 hours after the combined infusion. The quantitative estimation of insulin biosynthesis stimulated by high glucose levels was investigated in mice. The respective mice were treated with a single dose of STZ (200 mg/kg), ALXN (100 mg/kg), and N-nitrosomethylurea (78 mg/kg) by the IV route. The islets cells of the animals were

isolated after 10 min of drug administration. The rate of insulin biosynthesis in the islet homogenates was measured by the Immunoadsoprtion method using [^{3}H]-leucine as a marker. STZ and N-nitrosomethylurea partially inhibited insulin biosynthesis, whereas ALXN showed a complete blockage of insulin synthesis. The inhibitory effect was reversed by nicotinamide in STZ and N-nitrosomethylurea-treated homogenates. However, this inhibitory effect of nicotinamide was absent in the case of ALXN-treated homogenates. Guinea pigs produced Type-2 DM due to diet-induced glucose intolerance, further optimized by STZ. STZ alone could not produce hyperglycemia in guinea pigs for more than 21 days. However, STZ treatment after a high fat and carbohydrate diet produced persistent hyperglycemia for more than 3 weeks and insulin secretory activity resembling type-2 diabetes. It is reported that multiple low doses of STZ (40 mg/kg, IP, daily for 5 days) induce autoimmune diabetes in mice controlled by genetic H-2 complex (Major histocompatibility complex). The destruction of β cells can produce diabetes due to autoimmunity developed by NOD (Non-obese diabetic mice). It was found that STZ can protect and reverse autoimmune diabetes in young NOD mice, but this effect was not similar in the case of ALXN. This preventive effect was believed to be mediated by T cells and reversed with the treatment of anti-CD8 antibody.The effect of STZ (180 mg/kg, IV) and ALXN (75 mg/kg, IV) on the autoimmunity of mice in a transplantation model was investigated. STZ-treated animals showed a lesser number of splenocytes recovery than ALXN-treated animals. The recipient mice treated with STZ exhibited more prolonged survival (7 to 24 days) of rat islet grafts than ALXN-treated mice (6 to 7 days). In the cell lines HL60, K562, and C1498, STZ showed IC50 values of 11.7, 904, and 1024 µg/ml, whereas ALXN exhibited higher values of 2809, 3679, and >4000 µg/ml. These observations show that STZ can be accepted as more cytotoxic agents than ALXN *in vivo* and *vitro*. Moreover, STZ-treated animals observed lymphocytopenia, which may be the reason for the more prolonged survival of the rat islet grafts than ALXN-treated animals. The effect of STZ (150 mg/kg) and ALXN (50 mg/kg) on the immunity of diabetic mice caused defects in immune mechanisms. After insulin treatment, there was a complete and 70-80% reversal of impairment in ALXN-treated and STZ-treated animals, respectively. For 2 days, exposure to STZ (≥1 mmol/l) showed adverse effects on lymphoid functions, whereas no toxicity was observed in the case of ALXN (≥14 mmol/l) *in vitro*. However, both the agents caused a severe toxic effect on isolated islet cells. *In vitro* aberrations in the lymphocytic chromosomes were observed in STZ-diabetic mice, which were absent in the case of ALXN. Thus, it suggests selecting ALXN over STZ for investigating diabetic immunological mechanisms. STZ (55 or 100 mg/kg, IV) in rats and ALXN (100 or 200 mg/kg, IV) in rabbits were administered to investigate the influence of these agents on lipid profiles. Enzymatic kits were used to estimate the concentration and composition of total plasma and lipoprotein lipids. Animals treated with higher doses were only selected for these parameters. Cholesteryl ester transfer activity was determined by incorporating ^{3}H-cholesteryl ester into LDL in animals treated with high and low doses. In the case of STZ and ALXN, abnormalities in the lipid profile were independent of the cholesteryl ester transferase enzyme activity. It was also suggested that lipoprotein metabolism might be affected due to the severity of diabetes. The effect of a single dose of STZ (50 mg/kg, IP) and ALXN (150 mg/kg, IP) on the expression level of messenger RNA of transporters Mrp2, Mdr1, Oct1, and Oatp1 in rat liver was investigated by

RT-PCR (real-time PCR) method. STZ reduced mRNA expression of transporters Mdr1and Oatp1, while ALXN reduced mRNA expression of all four transporters. There may influence the effects and pharmacokinetics of related substrates of these transporters due to the reduced expressions caused by STZ- or ALXN-induced diabetes. Chronic diabetes induced by STZ (65 mg/kg, IV) caused severe changes in the heart muscles of rats. The changes in the ultrastructure of cardiac cells were observed 8 weeks after diabetes induction by using electron microscopy [10]. Nuclear level changes such as chromatin condensation and membrane folding were prominent. Other abnormalities such as mitochondrial swelling and matrix clearing were observed clearly. The lysosomal membrane started incorporating into the mitochondrial matrix with a significant increase in the number. The number of lipid droplets also increased markedly. Contraction of sarcomeres, degeneration of myofibrils, and separated intercalated discs were observed in the focal areas of the heart muscles. Any changes in the structure of endothelial cells or smooth muscles in smaller blood vessels and the appearance of atherosclerosis were not seen throughout the experiment. Hypertension is produced in rats suffering from diabetes induced by STZ (50 mg/kg) by intravenous route. When measured by the tail-cuff method, diabetic rats increased systolic pressure between 2-7 weeks compared to control groups. Other cardiac changes like bradycardia and narrowing of pulse pressure were also observed. Intraperitoneal administration of STZ (45 mg/kg) in 10 weeks old male mice for 5 days produced the highest level of blood glucose in 4^{th} weeks. An increase in blood cholesterol level was also observed in these diabetic mice. Heart aortic tissues were isolated on the 42^{nd} day for histopathological studies. Atherosclerotic plaques have appeared in these aortic tissues. The effect of STZ- (65 mg/kg, IP) and ALXN- (200 mg/kg, IP) induced diabetes on serum as well as tissue lysosomal enzymes (cathepsin D, cathepsin B_1, β-N-acetyl glucosaminidase, and β-glucuronidase) and collagenase activity in rats was investigated. Dermal collagenase activity was significantly higher in the STZ- and ALXN-treated groups than in normal. However, there was no significant difference in spleen and liver collagenase activity. In STZ-treated rats, renal collagenase activity was significantly lowered. Except for splenic β-N-acetyl glucosaminidase in STZ-treated rats, all other mentioned lysosomal enzymes were higher in liver, skin, and spleen in STZ- and ALXN-treated rats. Increased renal β-N-acetyl glucosaminidase and β-glucuronidase with unchanged cathepsin D and B_1 level was found in ALXN-treated groups. Renal lysosomal enzyme activity was lowered except for cathepsin D in ALXN-treated rats. The well-controlled glucose level in blood can protect against glomerulopathy in rats. Rats were made diabetic by STZ (90 mg/kg, IV) and divided into 3 groups well-controlled, poorly-controlled, and usual by maintained insulin treatment. Glomerular tissue sections were observed after 6 months. Glomerular basement membrane thickness was significantly more in poorly-controlled animals when compared to the other two groups. The carcinogenic effect of STZ (60 mg/kg, IV) and ALXN (40 mg/kg, IV) on renal cells of rats was investigated. After diabetes induction by STZ and ALXN, 130 out of 160 and 72 out of 100 rats survived, respectively. Rarely invasive epithelial malignant types of tumors were found in 3 out of 56 STZ-treated rats within 8 months. After 8 months of STZ treatment, 30.8 % (24 out of 74) of rats showed clearly visible tumors in the kidney as well as 2 of these infected rats showed tumors

in the lungs and liver. About 40 % of tumors in the kidney were found to be bilateral. No rats treated with ALXN produced renal tumors in the study period [11].

The effect on the male reproductive system of adult Wistar rats due to STZ- (30 or 60 mg/kg, IP) induced diabetes was studied. Testes and epididymis were isolated for histological examination after 8 weeks of STZ administration. Normal epididymal sperm morphology observed by computer-aided sperm analysis was impaired in both dose levels. In both dose levels, rat testes showed a decreased number of spermatozoa. Spermiogenesis was diminished in rats treated with STZ (60 mg/kg, IP). Diabetes was induced by injecting STZ (300 mg/kg, IP) and ALXN (300 mg/kg, IP) to investigate the effects of diabetes-induced by these two agents on the development of follicular and early embryos in mice. After 8 h of human chorionic gonadotropin (hCG) treatment, STZ and ALXN-treated animals showed significantly reduced breakdown of germinal vesicles compared to control groups. Insulin treatment *in vivo* reversed this maturation delay of oocytes in diabetic groups. The development of embryos (development rates and stages) was significantly impaired in STZ- and ALXN-treated mice compared to the control group. This effect was also improved by *in vivo* insulin treatment [12].

Dermatological changes associated with diabetes were investigated by treating a single dose of STZ (150 mg/kg, IV) and ALXN (45 mg/kg, IV) in hairless mice. Loss of water content in stratum corneum but not in transepidermal was observed in STZ- and ALXN-treated mice. Stratum corneum showed either high or normal amino acid with significantly reduced triglyceride content in mice treated with STZ. However, the cholesterol, fatty acid, and ceramide levels were alleviated in the stratum corneum of mice treated with STZ. STZ reduced the proliferation rate of basal cells with a larger area of corneocyte layers. However, marker proteins associated with the differentiation of the epidermis were unchanged. Rats treated with STZ (60 mg/kg, IV) showed qualitative and quantitative impairment in the dermal wound healing. There was a reduction in accumulation and extent of cross-linking of collagen in the wounded area. The healing process can be improved by administering insulin after the rats are wounded [5].

STZ and ALXN at low doses can change the neurochemistry of the rat brain without producing diabetes when administered by the intracerebroventricular (ICV) route. ICV STZ treatment can reduce glucose and energy metabolism in the rat brain. The long-term learning and memory activity of rats can be declined progressively by administering STZ by ICV route. Both STZ and ALXN by ICV treatment can lower the antioxidant capacity of the rat brain, making it susceptible to cognitive disorders. STZ- (70 mg/kg, IV) and ALXN-(160 mg/kg, SC) induced diabetic rat brain exhibits alerted expression of monoamine transporter mRNA depending on the duration of the disease. Dorsal raphe nucleus showed increased 5-HT transporter expression in STZ (4-8 weeks) and ALXN- (1-4 weeks) induced diabetic rats. Ventral medial bundle of STZ- (4-8 weeks) and ALXN- (1-4 weeks) diabetic rats decrease DA-transporter mRNA. Gene expression of NA-transporter was also markedly reduced in both STZ- and ALXN- diabetic rats [13].

Nonlinear male rats aged around 16 weeks were made diabetic by ALXN (300 mg/kg, IP) for investigation of effects on choroidea and retina. Pathological changes were studied in 30 and 60 days diabetic rats using light and electron microscopy. The thickness of the

retina and the number of choroidal blood vessels and melanocytes were reduced in the diabetic rats. Photoreceptors in the retina were destructed partially with edema in the interstitial spaces. Effects of long-term diabetes on the cornea and its nerve fibers of ICR mice strain were investigated by administering STZ (200 mg/kg) and ALXN (75 mg/kg) intravenously. Corneal tissues were isolated from 42 weeks aged mice after maintaining hyperglycemia for 35 weeks. Histological examination significantly reduced the density of terminal epithelial nerves, subbasal nerve plexuses, and intraepidermal nerve fibers. Both STZ- and ALXN-treated mice showed smaller mean sizes of axons of sural nerve when compared to expected. The effects of diabetes on the pineal gland of domestic juvenile pigs were studied by administering STZ (150 mg/kg, IV). Biochemical estimation of pineal gland contents was done after 6 weeks of STZ treatment. Functions of the pineal gland were affected, characterizing altered levels of neurotransmitters and their metabolites. The ultrastructural and histological changes of the submandibular salivary gland caused by diabetes were investigated in albino rats treated with intraperitoneal ALXN (140 mg/kg). Submandibular salivary glands were isolated after 6 weeks of ALXN treatment to examine defects produced. Marked alteration in glandular tissues, presence of vacuoles in intracytoplasmic, changes in shape and size of acinar cells, eosinophilic granular convoluted tubules, and atrophic striated ducts were observed at the histological level. At the ultrastructural level, acinar cells and ducts of diseased rats produced cytoplasm with large vacuoles, perinuclear spaces with dark nuclei, degenerated mitochondria, and rough endoplasmic reticulum. Moreover, nuclear chromatins clump in myoepithelial and serous cells with reduced secretory vesicles [14].

DISCUSSION AND CONCLUSIONS

Researchers have continuously been trying to understand the pathogenesis and various complications associated with diabetes. Various natural or synthetic agents have been screened to get a satisfactory solution for the management and treatment of diabetes. Several animal models have been developed for detailed study of diabetes. To be considered an ideal model for diabetes in rodents, it should imitate the pathogenesis and complications of human diabetes either *in vivo* or *in vitro.* Chemical models are mostly preferred over genetic or surgical methods due to the lower rate of morbidity and mortality for induction of diabetes. Chemicals produce diabetes in rodents, explicitly destroying β cells of pancreatic islets and/or inhibiting insulin synthesis or action. Diabetes intensely damages the liver, which maintains lipid and glucose homeostasis. Diabetes can alter lipid profile by directly or indirectly affecting the regulatory organs like the liver and kidney. Diabetes is also responsible for causing various vascular complications such as myocardial disease. Chronic diabetes can severely affect reproductive efficiency both before and after fertilization.

Streptozotocin is more preferred than Alloxan as a diabetogenic agent. This is because of some advantages associated with the induction of diabetes by streptozotocin treatment. Before and after the treatment, STZ shows more stability than Alloxan in solution form since it has a half-life of 15 minutes which is longer than Alloxan (1.5 minutes). STZ produces diabetes for about 3 months, but Alloxan can do the same for less than one month. More specific to cellular toxicities, Alloxan shows higher mortality in experimental animals than STZ. Alloxan can produce systemic toxicity by attacking GLUT2 expression of the cells other than B cells, but STZ has

more selectivity towards B cells of pancreatic islets. Moreover, blood glucose levels cause less interference in causing diabetes in experimental animals.

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Conflict of interest

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Reference

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