



REDISCOVERED THE INDUCTION OF DIABETOGENIC AGENTS IN THE EXPERIMENTAL ANIMAL MODEL: REVIEW

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ABSTRACT: Type- I & II Diabetes is mainly attributed to the rapid rise in an alarming unhealthy a serious threat to humankind's health at life style. Diabetes is a metabolic disorder, whose prevalence has continuously increased worldwide over the past few decades. Therefore, Experimental studies have demonstrated animal models play a vital role in understanding such a disease, which is treatable only. Alloxan and Streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their Alloxan and Streptozotocin action has been intensively investigated and located to be selectively harmful to pancreatic β cells because it preferentially accumulates within the β cells as glucose analogues now is quite well understood. The remarkable discovery that a single injection of diabetogenic agents, a large number of pharmacological agents and animal studies are used in examination of diabetes for understanding the pathogenesis, complications, genetic and environmental influences. However, this review may provide an integrated view of their interaction with diabetogenic metabolic action, fast start, complications and their precaution investigated in the experimental study.

Abbreviations: STZ, Streptozotocin; IDDM, Insulin dependent diabetes mellitus; NIDDM, Non insulin dependent diabetes mellitus; ATP, Adinocin tri phosphates; TUNEL, Terminal deoxynucleotidyl transferase; HCL, Hydro chloric acid; NaCl, Sodium Chloride; H₂O₂, Hydrogen peroxide; GSH, Reduced glutathione; ROS, Reactive oxygen species; Ang, Angiotensin; NADPH, Nicotinamide adenine dinucleotide phosphate; SOD, Superoxide dismutase; GLUT2, 2-glucose transporter; DNA, Deoxyribonucleic acid; ADP, Adinocin diphosphate; Ca²⁺, Calcium 2 Ions; IUPAC, International Union of Pure and Applied Chemistry; GlcNAc, glucose and N-acetyl glucosamine; MNU, N-methyl-N-nitrosourea; NAD⁺, Nicotinamide adenine dinucleotide; cGMP, Cyclic guanosine mono phosphate; PARP, Polymerase gene are resistance to pancreatic; MDA, Malonaldehyde; TxB₂, Thromboxane-B₂; TxA₂, Thromboxane-A₂.

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INTRODUCTION

Type I & II Diabetes is mainly attributed to the rapid rise in an alarming unhealthy a serious threat to mankind health at life style, It is actually a disorder carbohydrate, fat, and protein metabolism caused due to abnormal utilization (or) production of insulin deficiency inhibitory action, characterized by high blood glucose levels occurred in the body can lead to hyperglycemia (or) hyperglycemia (Kitabchi et al., 2009; Diabetes Blue Circle Symbol., 2006). This can be causes various types of secondary complications damage to eyes, kidneys, nerves, heart and blood vessels associated with morbidity and mortality (Kruger et al., 2012). These areas under discussion, we are all known, many researchers suggested them. And however, in animal model during experimental work on the induction of Diabetes mellitus very difficult and has been occurring many complications (Kitabchi et al., 2009). Induction of diabetes mellitus in animals two types of diabetogenic agents available in pharmacy. Such as i) Streptozotocin (STZ), ii) Alloxan. Diabetes mellitus (IDDM and NIDDM, respectively) can easily be induced in animals by using diabetogenic agent's at intravenous or intraperitoneal injection method. This may provide an integrated view of their chemical properties and biological effects (Mendez et al., 1994; Etuk et al., 2010).

Before induction of diabetogenic drugs some known practically accepts

Induction of diabetes in animal species Alloxan and STZ has proven to be better diabetogenic agents most effectiveness and greater reproducibility toxins. For injection in experimental animals and for optimum results, it's best to be administered at starvation state and newly prepared, dissolved in citrate buffer (pH 4.4-4.5) (Chinedum Ogbonnaya Eleazu et al., 2014). Fasting may be a common procedure for animals in experiments. Although fasting is also necessary for maintaining scientific reasons and actions. Fasting causes sever changes within the physiological and biochemical process of the animal that become additional serious with longer duration of food withdrawal (Gannon et al., 1997).

However, additionally the often-used fasting periods of 16-24 hours bring about vital changes, which can significantly effect on the responsiveness towards experimental stimuli.

Fasting can be expected to cause, among alternative things, stress, aggressive behavior as well as reduction in body weight, body temperature, cellular dehydration, intake of fluids and plasma glucose levels (De Leo; 2010; Gannon; 1997). If researchers think about it necessary to quick their animals in order to empty portions of the gastrointestinal track. Once the animals are most active- significant reduction in liver weight and glycogen count, as well as increases in levels of glycerol, free fatty acids and acetoacetate have been measured after 3 hours cites a number of studies that show increases in plasma levels of glucose, urea, lactate and amino acids, a variety of hormones together with insulin nervous system (Fell et al., 1980). It is additionally important to be aware that fasting can lead to reduced basal metabolic rate, which may persist when food has been re-introduced, while body weight is returning to a normal range. Animals that were fasted are showing increased locomotory behavior and an increase in the time spent grooming, which also resulted in the accumulation of a great quantity of hair within the stomach (Herrera et al., 1981). Shorter period of deprivation resulted in smaller changes in the animal's physiology and behavior (Kmiec; 2006; Herrera; 1981). This might be attributed to the fact that after administration of diabetogenic agents causes a decrease in hepatic glycogen at intervals 24-72 hours because of its conversion to anionic radicals and pancreatic destruction, lead to IDDM and NIDDM respectively (Cominacini et al., 1995). Moreover, the Alloxan and STZ model mimics many of the acute and chronic complications of animal disease model when compared to human diabetes and given the stable similarities of some of the structural, functional and biochemical abnormalities to human disease; it is an assign model to assess the mechanism of diabetes.

Showing the table 1, some researchers experimentally proven that, mainly experimental animals were using induction of diabetes was associated with Alloxan are mixed in 0.9% NaCl buffer and also STZ are mixed with 0.1M citrate buffer solution may be better to administrated. And some experiments optional that rat, rabbit, mice, dogs, guinea pig, monkey, hamsters also were used.

Diabetes Stages with induction of Alloxan and Streptozotocin

One of the most important diabetogenic agents of Alloxan and STZ has been commonly employed as an experimental design in animal models. Both are cytotoxic glucose analogues. That has induced a multiphasic blood glucose response and accompanied by corresponding inverse changes within the plasma insulin concentration. So sequential ultrastructural β cell changes finally it will be lead to can occur necrotic cell death cause insulin dependent diabetes mellitus in experimental animals (My thili., et al 2004; Lenzen et al., 2008). After administration of diabetogenic agents at the intravenous or intraperitoneal injection method- circulate in the body as twenty percent of the drug is metabolized and excreted by the kidney. The first stage that comes into prospect within the first minutes from continuing for 30 minutes, this short-lived transient hypoglycemic response is noted to the evolution of a transient stimulation of insulin fount inception, by an increase of the plasma insulin concentration (Lenzen et al., 2008). Therefore, due to the action of diabetogenic agents may be inhibition of hyperinsulinemia uptake into glucose phosphorylation attributed to glucokinase activity through a temporary rise in ATPs (Kliber et al., 1996).

Thus, the second stage is hyperglycemia. During this condition starts to increase in blood glucose concentration, whereas at the same time plasma insulin concentration decrease appearing one hour when administration of diabetogenic agent injections (Wrenshall et al., 1950). This pronounced fist hypoglycemic condition typically last 2-4 hours. Morphologically during this method inhibition of insulin secretion from pancreatic β cells led to hypoinsulinaemia (Goldner et al., 1944). These characteristically disturbed pancreatic β cells show intracellular vocalization, dilation of the rough endoplasmic reticulum, other cell organelles, including swollen mitochondrial loss of Christie has a number of secondary granules in the inner membrane. The observations are consistent with a defective of mitochondrial energy production. Due to the action of the Alloxan & STZ an inhibition of pre-proinsulin biosynthesis packaging, storage of insulin in the secretory granules processing their β cell toxicity (West et al., 1996; Lenzen et al., 2008). The 3rd phase hypoglycemic typically 4-8 hours after diabetogenic agent injection. Which can the blood glucose response is one more a hypoglycemic stage lasts for several hours; it should be severe cause's convulsion (Tasaka et al., 1988). This severe transmutation hypoglycemia is the results of a flooding of the circulation with insulin as a result of the toxic induced secretory granule and plasma membrane rupture. In additionally, alternative organelles are damaged that include cisternae of rough endoplasmic reticulum and also Golgi apparatus. Moreover, mitochondria loose structural integrity in this particular phase, in these ways that attributed to inner and outer structural changes are irreversible and extremely characteristic for a necrotic cell death of pancreatic islets. In addition to the morphological changes seen within the first phase, the β cell nuclei are pyknotic and show no TUNEL-positive staining changes are irreversible (Jacobs et al., 1937). On the 4th stage occur permanent diabetic hyperglycemias conditions throughout morphological and ultra structural analyses complete degranulation and loss of the perfection of the β cells within 24-48 hours after injection of diabetogenic agents (Srinivasan et al., 2007). No- β cell such as alpha cells and alternative endocrine and non-endocrine islet cell varieties as well as the extra pancreatic parenchyma remain intact proving the β cell selective character of the cyanogenetic action of these diabetogenic compounds, (Banerjee et al., 1948) Cell debris of the dying β cells is removed by scavenger macrophages, that don't seem to be activated. After the destruction of the β cells, when survival through insulin supplementation is secured, so-called end stage islets reside within the pancreas exclusively composed of non- β cells. As it has been widely Injections of Alloxan- All of the described morphological features of β cell dysfunction are constitutional native of necrotic cell death will occur diabetes (Lenzen et al., 2007). See figures 1, 2, 3 & 4 pancreas tissue Hematoxylin and Eosin stain. (Retrieved by Ref. 51 & 59).

Alloxan

Alloxan is a carcinogen and cytotoxic glucose analogue (Lenzen et al., 1988); it can be helpful to induction of diabetogenic agent in experimental diabetes in animal models such as rabbits, rats, mice and dogs with completely different grades of disease severity by varying the dose of Alloxan used (See Table 1) (Etuk et al., 2010; Nagy et al., 2014). Alloxan has a strong oxidizing agent, its molecular formula of $C_4H_2N_2O_4$, molecular mass of 142.06 g/Mol (See figure: 5). Alloxan is a hydrophilic and unstable substance. Its half-life at neutral pH and 37 °C is about 1.5 min and is longer at lower temperatures (Bolton et al., 1964; Lenzen et al., 2007). Alloxan is a urea derivative that causes selective necrosis of the β - cells of pancreatic islets, and Rapid uptake by insulin-secreting cells has been proposed to be one of the vital features determining Alloxan diabetogenicity (Iranloye et al., 2011). Alloxan is a simple nitrogenous organic compound. It has been better known for more than from 150 years ago; the scientist Brugnatelli was originally isolated Alloxan in 1818. Then, in 1838 Alloxan was discovered by the fathers of this science, Frederick Wohler and Justin J. Liebig synthesized an oxygenated pyrimidine urea derivative (Wohler et al., 1838). Those associated with uric acid and the naming of some 13 derivatives of uric acid, including Alloxan. Which they later, namely is called Alloxan in 1943. Additionally, induction of diabetes with Alloxan in animal model at Rabbit first described by the scientists Dunn, Sheehan and McLetchie in 1943 (Dunn et al., 1943). Alloxan chemically associated IUPAC nomenclature 2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6- Pyrimidinetetrone.

Chemical properties of Alloxan

1. An oxygenated pyrimidine derivative. It is also a barbituric acid derivative (5-ketobarbituric acid)
2. Present as Alloxan hydrate in aqueous solution.
3. A β cell toxic glucose analogue with a molecular shape similar to that of glucose.
4. A very hydrophilic compound (partition coefficient -1.8).
5. A weak acid and toxic thiol reagent.
6. For experimentation concentrated stock solution in 0.01 M HCL, kept on ice, should be used and added to test medium just period to the start of the experiment in order to obtain the final concentration.
7. For injection stock solution should be diluted with ice cold saline (0.9% NACL).
8. It is reduced to dialuric acid in the presence of GSH and other thiols.
9. During each redox cycle a mild quantity of "compound 305", an Alloxan - GSH adduct of unknown structure with a characteristic absorbance at 305nm that is not toxic, is formed.
10. A protoxin, with generates in its xenobiotics metabolism, toxic ROS species (superoxide radicals (O_2^-)), hydrogen peroxide (H_2O_2), when it redox cycles with dialuric acid.

Alloxan Mechanism of Action

Alloxan exerts its diabetogenic action when it's administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of Alloxan who wanted to for induction of diabetes depends upon the animal species (See Table 1). Alloxan is a very unstable chemical compound with a molecular shape similar to glucose. The mechanism of Alloxan action has been attributed to evoke a sudden rise in blood insulin secretion in the presence or absence of glucose (Szkudelski et al., 1998; Iachin et al., 2012). That appeared simply after Alloxan injection was also observed in vivo just after Alloxan injection to animals. The action of Alloxan in the pancreas is progression by its swift uptake by the insulin secretion B cells that have been proposed to be one of the vital roles determining Alloxan diabetogenicity. It selectively inhibits glucose-induced insulin secretion effect of Alloxan through specific inhibition of glucokinase, the glucose sensor of the β cell, and it causes a state of insulin-dependent diabetes mellitus (Weaver et al., 1978; Boquist et al., 1983). The reduction process occurs within the presence of pancreatic β cells totally different reducing agents like, reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups are very susceptible to its action (Elsner et al., 2006). Alloxan reacts with high affinity containing two -SH groups of the cellular sugar binding compounds of glucokinase leading to the formation of the disulfide bond and inactivation of the enzyme. Glucose can inhibit glucokinase conversely the inactivation hindering the access of Alloxan to the -SH groups of the enzyme. And additionally SH-containing compounds essential for proper glucose-induced insulin secretion is glucokinase as a result of Alloxan reduction dialuric acid formed, then re-oxidized back to, establishing a redox cycle for the generation of superoxide radicals and reactive oxygen species (Grankvist et al., 1979). Another biological effect of Alloxan is endocrine pancreatic β cell toxicity and diabetogenicity that will be attributed to Alloxan-induced due to the action of insulin dependent redox cycling and ROS generation. Alloxan can generate 'reactive oxygen species' (ROS) in a cyclic reaction between this substance and its reduction product, dialuric acid (Yano et al., 2004). The β cell toxic action of Alloxan is initiated by free radicals formed in this redox reaction. During each typical redox cycle a small amount of 'Compound 305', an Alloxan-GSH adducts are formed. The intracellular concentration of Compound 305 increases in a time dependent manner, which gradually decreases the amount of reduced GSH obtainable within the cell for redox cycling, thus producing a lower pro-oxidative ratio between Alloxan and GSH, rather than a higher antioxidative ratio (Bromme et al., 2002). ROS are involved in many of the angiotensin II (Ang II) signaling pathways. Angiotensin II stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity via the Angiotensin I (Angiotensin I) receptor to produce the superoxide anion, H_2O_2 and hydroxyl radicals (Griendling et al., 2000). And that Cytochrome C is dissociated from the inner mitochondrial membrane response to cardiolipin peroxidation by reactive oxygen species (ROS) prior to the re-lease into the cytosol (Takuya Ichimura et al., 2011). Autoxidation of dialuric acid generate superoxide radicals and undergo dismutation to yield hydrogen peroxide (H_2O_2) within the presence of superoxide dismutase (SOD). As a result, extremely reactive hydroxyl radicals are formed according to the Fenton reaction within the presence of ferrous and H_2O_2 . It can be the Fenton reaction in the presence of an appropriate metal catalyst, to produce the highly injurious hydroxyl radicals (Takahara et al., 1952).

The Autoxidation of dialuric acid involves the intermediate formation of the cell requires the presence of an appropriate tool, typically the tripeptide glutathione (GSH) to generate the oxidation-reduction cycling partner, dialuric acid, and oxidized glutathione (Rerup et al., 1970). Normally the ability for Alloxan reduction, redox cycling and the generation of ROS within the circulation isn't sufficient to proscribe the Alloxan molecule from reaching and entering the β cell (Sakurai et al., 1989). However, other reducing agents proposed that one of the SH-containing compounds important for proper glucose-induced insulin secretion is glucokinase, being very vulnerable to Alloxan (Zhazng et al., 1992). Alloxan inhibits several cellular functions at higher concentrations such as the ability to oxidize thiol groups of many functionally necessary enzymes like hexokinase, phosphofructokinase, calmodulin-dependent protein kinase, aconitase and different proteins (Gorus et al., 1982). In addition, due to their hydrophilic character, both Alloxan and glucose don't penetrate the lipid bilayer of the cell membrane attributed to accepting that the GLUT2 glucose transporter molecule interaction of the necrotic death β cell (Weaver et al., 1978). However, it has been argued that glucose counteracts Alloxan cytotoxicity in vitro and in vivo. Alloxan does not block the action of the GUTR2 transporter inside the biological lifetime of the chemical compound (Takasu et al., 1991). The cell groups of protein generate ROS on the effect of DNA of pancreatic islets. The fragmentation of DNA takes place within the β cells exposed to Alloxan that causes DNA damage, which stimulates poly ADP-ribosylation, a procedure participating in DNA repair. Antioxidants like superoxide dismutase, catalase and the non-enzymatic scavengers of hydroxyl radicals have been found to protect against Alloxan toxicity (Ledoux et al., 1988). In addition cytosolic free elevated Ca^{2+} has conjointly been accounted to constitute an important step in the diabetogenic action of Alloxan. The calcium influx results from the flexibility of Alloxan to open voltage dependent calcium channels and increases calcium innovation into pancreatic cells. The increased concentration of Ca^{2+} ion further contributes to the supraphysiological insulin secretion that along with ROS finally causes impairment of β cells of pancreatic islets (Park et al., 1995).

Streptozotocin

From that time of discovery Streptozotocin (STZ) has been one of the chemical agents for the induction of diabetes in experimental animal model (Rat, Mice, Rabbit and Dog etc...) (Rerup et al., 1970). It's a naturally occurring compound, produced by the soil bacterium *Streptomyces achromogenes*, which exhibits a broad spectrum of antibacterial properties. It is a mixture of α - and β -streamers that seem as pale yellow or off-white crystalline powder (Elsner et al., 2000). STZ is a cytotoxic glucose analogue, naturally occurring chemical that's notably deadly toxic to insulin, and cause a state of insulin dependent diabetes mellitus through its capacity to induce a selective necrosis of the pancreas β cells. STZ and is widely want to generate animal models (Rat, Mice, Rabbit and Dog etc..) of diabetes (Takassu et al., 1991; WHO 1980). Streptozotocin doesn't have an effect on the pancreatic β cells of humans when utilized in the treatment of islet-cell carcinomas and malignant carcinoid tumors in humans (Mithyli et al., 2004). This resistance of the human β cells to STZ is attributed to the very low level of the constitutive GLUT 2 transporter expression within the human β cell (Ryan et al., 2002). Streptozotocin incorporates a formula of $\text{C}_8\text{H}_{15}\text{N}_3\text{O}_7$, molecular weight of 265 g/Mol (See figure: 6) and the structure consists of nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other end (Ross et al., 1967). STZ chemically associated IUPAC nomenclature 2-deoxy-2-(3-(methyl-3-nitrosoureido) -D-glucopyranose).

STZ has a structure similar to glucose (Glu) and N-acetyl Glucosamine (GlcNAc) (See Fig.1). STZ is taken up with pancreatic β -cells via the GLUT 2 transporter where it causes β -cell death by DNA fragmentation due to the nitrosourea moiety (Chinedum Ogbonnaya Eleazu et al., 2014).

Chemical properties of Streptozotocin

1. It is a hydrophilic compound.
2. It is a Glucosamine derivative.
3. A cytotoxic methylnitrosourea moiety (N-methyl-N-nitrosourea) attached to the glucose molecule.
4. A β cell toxic glucose analogue.
5. An alkylating agent.
6. Relatively stable at pH 7.4 and 37°C.
7. For injection, a stable solution in citrate buffer (pH 4.5) is most suited.
8. For in vitro experimentation focused stock resolution in 0.01M HCL, kept on ice, should be used and added to test medium just prior to the start of the so as to get the ultimate concentration.
9. Storage: -20°C.

Streptozotocin Mechanism of action

Streptozotocin is a toxic chemical compound. The frequently used single Endovenous or intraperitoneal multiple dose (See Table 1) in animals to induce diabetes mellitus and ketoacidosis-resistant diabetes mellitus severally. Diabetes (IDDM and ketoacidosis-resistant diabetes mellitus, respectively) can easily be induced in animals was first described by Portha 1974 (Portha et al., 1974). Totally different doses are evoked, such treatment is employed predominantly within the animals and the induction of diabetes is mediated by the metabolic activation of the immune system via complete adjuvant prior to reduce free radical in the animals. After two hours STZ injection refers to the hyperglycemia is observed with a B cell is accompanied by alterations in blood insulin and glucose concentrations. About 6-8 hours later, symptom happens with hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia evolves and blood insulin levels decrease (Rerup et al., 1970). This hyperglycemia inducted with STZ experimental animals, tend to possess reflect abnormalities in B cell function; causes a insulinopenia state of pancreas, renal, liver injury, and intestine being consistently increase than those within the plasma glucose. STZ additionally causes cardiac and adipose tissue damage and increases aerobic stress, inflammation, endothelial dysfunction that is followed by its permanent loss and cells are square measure broken (Valentovic et al., 2006). In mammalian cells, the mechanism of action of STZ that results in cell death wasn't fully identified; the selective pancreatic β cell toxicity and diabetic condition, resulting from STZ induction-that is related to the glucose moiety and also the action of low affinity GLUT2 glucose transporter into the β cells enter within the cell wall (Schnedl et al., 1994; Robert et al., 2001). A reduced expression of GLUT2 has been found to prevent the diabetogenic action of STZ determined that STZ it restricts GLUT2 expression in vivo and in vitro when administered in multiple doses (Wanz et al., 1995).

Therefore the toxic action of STZ involves and a chemically related alkylating compound requires their uptake in to the cells harm, the alkylating activity of STZ is expounded to its ethylating agents N- ethyl-N-nitrosourea and ethyl methanesulphonate moiety (Delaney et al., 1995).The fact that N- ethyl-N-nitrosourea and ethyl methanesulphonate are significantly less toxic to insulin producing cell then MNU (N-methyl-N- nitrosourea) and methyl methanesulphonate has been taken as support for the contention that insulin producing cell, like in alternative cell varieties. Especially N- ethyl-N-nitrosourea and ethyl methanesulphonate has been attributed to O⁶-ethyl- guanine being less toxic then O⁶-methylguanine (Bennett et al., 1981). Though it was absolutely, thought to be a result of DNA and chromosomal damage one of the administrative division by mechanisms involving free radical generation throughout the STZ metabolism (BolZan et al., 2002). Another possible mechanism of the diabetogenic action of Streptozotocin that leads to necrobiosis cell death has been assign to its capacity to act as nitric oxide donor such as sodium nitroprusside or 3-morpholiniosydnonimine in pancreatic cells (Tiedge et al., 1999). Nitric oxide production in vivo in rat pancreatic β cell was determined early (about 2h) after STZ administration (Wada et al., 2004). The participation of nitric oxide in the cytotoxic effect of STZ was confirmed in many experiments Pancreatic B cells, Nitric oxide causes convincingly supported by the observation in PARP (Polymerase gene are resistance to pancreatic) are resistant to β cell death attributed by STZ in spite of acts as an inhibitor of several mitochondrial enzymes, DNA damage prevents the depletion of the co-factor NAD⁺ (Nicotinamide adenine dinucleotide) and the consequent loss of ATP (Burkart et al., 1999). Nitric oxide acts as an inhibitor of many mitochondrial enzymes, exposed to Streptozotocin has been shown manifested changes characteristic for nitric oxide action increased activity of guanyl cyclase and enhanced formation of Cyclic guanosine monophosphate (cGMP) (Turk et al., 1993). Nitric oxide reaction with super oxide anion results formation of peroxinitrate, which decomposes and produces other ROS, such as like hydroxyl group radicals. However, the results of several experiments provide the proof that nitric oxide isn't the only molecule responsible for the cytotoxic result of STZ (Hallwell et al., 1994). As a result of STZ administration, Oxidative stress is defined as an associate imbalance between the pro-oxidants and antioxidant defense system of the body as a result of steady state reactive oxygen species (Atalay et al., 2002). Oxidative stress has recently been shown to be responsible, a minimum of partially, for pancreatic β -cell dysfunction caused by glucose toxicity in hyperglycemia (West et al., 2000). Several reaction mechanisms are thought to be involved in the genesis of oxidative stress in both diabetic patients and diabetic animals and they include glucose auto-oxidation, protein glycation, formation of advanced glycation products and therefore the polyol pathway. Throughout these processes, ROS are produced and cause tissue damage (Hunt et al., 1990; Matsuoka et al., 1998). STZ treatment causes important increase in malonaldehyde however decreases inhibitor enzymes such as: antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase activities when compared with management animals in experiments. Decreases in antioxidant activities, and simultaneous increases in malonaldehyde (MDA) activities, indicate the susceptibility of pancreas to STZ's induction of oxidative stress (Gul et al., 2002; Henriksen et al., 2002). STZ can take part in production of superoxide anions and hydroxyl radicals; however, their role in triggering diabetes is negligible. On the other hand, this initial transient hypoglycemic phase is not determined within the case of a STZ injection, variance from Alloxan, STZ doesn't inhibit glucokinase. During these initial five minutes after toxic exposure, the β cells show no morphological sings of harm and /or damage (Lenzen et al., 1991). Involvements of Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and generated by exogenous sources such as ionizing radiation. This progress augmented of ROS by numerous pathways under diabetic conditions (Katalin et al., 2006). Growing evidence suggests that one vital involvement of ROS throughout STZ metabolism is that the production of uric acid as the final product of ATP degradation by xanthine oxidase from hypoxanthine. This reaction generates ROS such as superoxide and hydroxyl group radicals emanating from H₂O₂ disputation throughout hypoxanthine metabolism, accelerating the process of β cell destruction. This is coupled with the fact that the pancreatic β cell is devoid of catalase and glutathione peroxidase. The hydrogen peroxide subsequently generates free radicals like O₂⁻ and OH⁻ (Zamocky et al., 2008). These reactive compounds will cause peroxidation of lipids, resulting in the formation of hydroperoxy fatty acids and endoperoxides. This increases the formation of malonaldehyde and thromboxane-B2 (Tx_{B2}). The accumulation of Tx_{B2} along with thromboxane-A2 (Tx_{A2}) can cause platelet aggregation and promote thrombosis (Pushparaj et al., 2000). Increased ROS production has also been documented to inhibit aconitase which protects mitochondrial DNA (mtDNA) from degradation (Vergani et al., 2004).

Table-1: Different kind of Alloxan and STZ dosages associated induction of Diabetes.

Name of the animal	Alloxan dose (mg/kg)		Streptozotocin dose (mg/kg)	
	Low dose	High dose	Low dose	High dose
Rat	90-140	150-200	40-50	70-80
Mice	50-60	70-80	30- 40	50-70
Rabbit	80-90	100- 120	50-70	80-100
Hamster	40-60	80-100	40-50	60-70
Guinea pig	70-80	100-120	60-70	100-120
Dog	40- 60	60-70	50-60	80-100

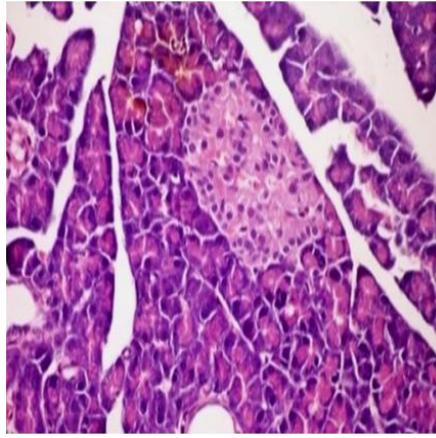


Figure-1: Rat- Normal pancreas tissue Hematoxylin and Eosin stain.

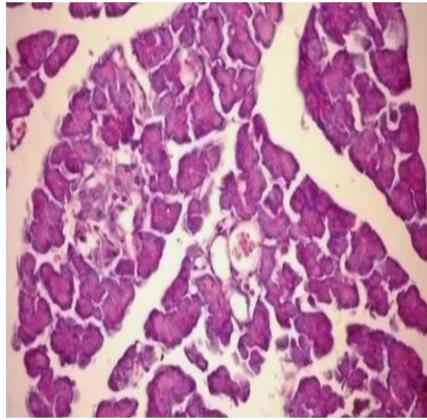


Figure-2: Alloxan induced rat pancreas tissue Hematoxylin and Eosin stain.

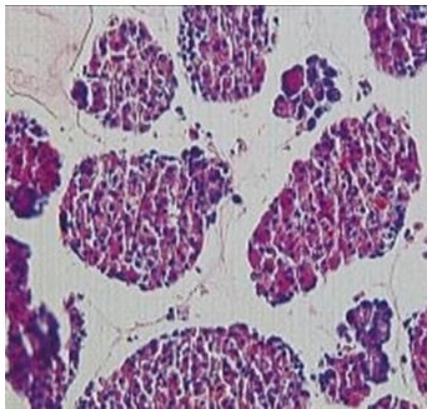


Figure-3: 0 min STZ induced rat pancreas tissue exposure Hematoxylin and Eosin stain.

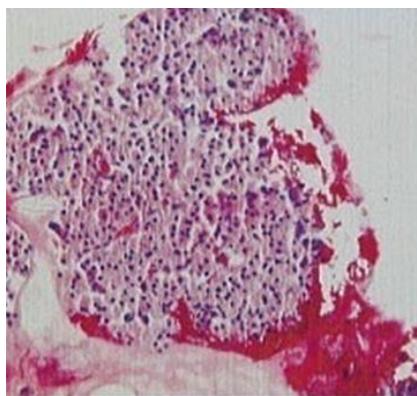


Figure-4: STZ induced- after 30 min rat pancreas tissue exposure Hematoxylin and Eosin stain.

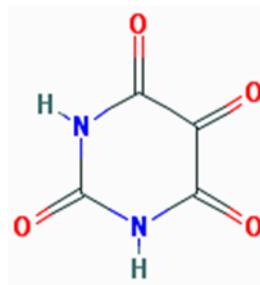


Figure-5: Alloxan

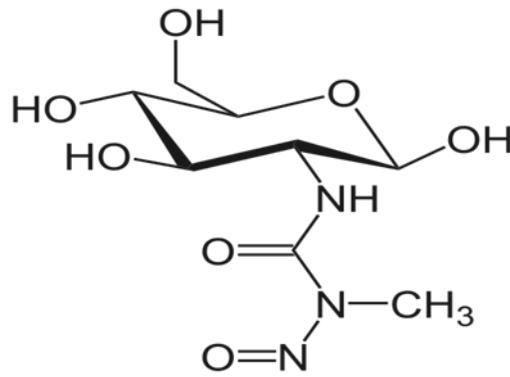


Figure-6: Streptozotocin (STZ)

To protect and prevention the diabetic animals from mortality

Induction of diabetes may be easily induced to the animals. Induction of diabetes by using diabetogenic agents in animals were associated standard diabetic blood glucose levels can be up to 250-350 mg/dl is better to done above 8 weeks experimental work (Liu et al., 1999). However, the induction of diabetes animals are protection and prevention from mortality can be complicated. Because imbalance of glucose and/or insulin regulation condition occurs, animals will be dying. During this approach, after induction of diabetogenic agents 24-72 hr (including the first night after diabetogenic agent's treatment) tends to blood glucose levels are 400mg/dl above the top end limit of portable glucometer will be complicated and It is possible for the animals to die (Joo et al., 2010). Thus, in generally- in diabetic patients may be take treatment for using minimum 40 IU of insulin injection daily (Patel et al., 2012). In this way, diabetes induced animals' protection and prevention from mortality may highly recommended injection of very small dose of insulin 1-3 units every 3 or 4 days (if BGL exceed 400mg/dl). It's important to monitor the glucose every 5hrs after injection in order to check for hypoglycemia (Atalay et al., 2002; American Diabetes Association., 2004). This allowed me that rats do not suffer a death by dehydration. Injection of low dose of insulin will not affect hyperglycemia but prevent ketoacidosis and maintain the rats in healthy condition. Generally discusses, you should not have difficulty in keeping STZ-diabetic rats alive for 1-2 weeks even without insulin injections (Qinna et al., 2015). This allowed me that rats do not suffer a death by dehydration. This produced about no complication in animals for a period of time limited up to 30-45 days long as the experiments lasted (Hughes et al., 2001).

Summary

Alloxan and Streptozotocin is a one of the most vital role in diabetogenic agent widely used in experimental in understanding such a diabetic disease in animal model. Both Alloxan and Streptozotocin cytotoxic effect was induces insulin deficiency. The foremost diabetogenic drug-induced diabetic model is the diabetes that is capable of inducing IDDM diabetes mellitus and leads to NIDDM respectively in experimental animals. The remarkable discovery that a single injection of Alloxan can produce diabetes mellitus in laboratory animals was made in 1942, in Glasgow, by John Shaw Dunn, Sheehan and Norman McLetchie. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide free radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter extremely reactive hydroxyl radicals are developed by the Fenton reaction. The activity of reactive oxygen species with an efficacy massive enhance in cytosolic calcium concentration by the action rapidly destruction of β cells. Therefore, Streptozotocin enters the β cell via a glucose transporter (GLUT2) and effort alkylation of DNA. DNA destruction induces activation of poly ADP-ribosylation, a role that is more vital procedure for the diabetogenicity of Streptozotocin than DNA damage itself. Poly ADP-ribosylation conducts to depletion of cellular ability to inhibit NAD⁺ and ATP. Enhanced ATP dephosphorylation after Streptozotocin intervention supplies a substrate for xanthine oxidase consequently in the formation of superoxide radicals. Accordingly, hydrogen peroxide and hydroxyl free radicals are also generated. Furthermore, Streptozotocin releases free toxic amounts of nitric oxide that suppress aconitase activity and participates in DNA damage. As an evolution of the Streptozotocin action, β cells undergo the destruction by necrosis. The surgical and genetic processes of diabetes induction are associated with a high rate of animal morbidity and mortality. So, efforts should be made in the direction of upbringing and uplifting the model of Alloxan induced diabetes mellitus in the experimental animals.

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