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Review

Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies

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ABSTRACT

Glycemic homeostasis refers to glucose balance or control within circulation in living organisms. It is normally and largely compromised in diabetes. The compromise when exacerbated, leads to several complications including retinopathy, nephropathy and neuropathy which are collectively known as diabetic complications and are the principal actors in co-morbidity and eventual mortality often associated with diabetes. The ability of therapeutic compounds including medicinal plants to restore glycemic balance or homeostasis in hyperglycemic condition is an index of their antidiabetic function and relevance. Alloxan and streptozotocin are the most popular diabetogenic agents used for assessing the antidiabetic or hypoglycemic capacity of test compounds. Notably, alloxan is far less expensive and more readily available than streptozotocin. On this ground, one will logically expect a preference for use of alloxan in experimental diabetes studies. Surprisingly, a sub meta-analysis of randomly selected studies conducted within the last one and half decade revealed otherwise. This observation necessitated the review of alloxan as a diabetogenic agent in animal studies.

1. Introduction

Alloxan which is chemically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analog [1]. The compound has the molecular formulae, $C_4H_2N_2O_4$ and a relative molecular mass of 142.06. Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving diabetes. Among the known diabetogenic agents which include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load and anti-insulin serum; alloxan and streptozotocin (STZ) are the most widely used in diabetes studies. The current average cost of one gram of

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alloxan and STZ are respectively 1.5 and 200 US dollars respectively. Due to relative affordability and availability, one will logically expect that alloxan will be more used compared to STZ [2]. However, a literature survey and sub meta-analysis that we carried out on the use of both compounds in experimental diabetes studies conducted within the last one and half decade (2000–2016) suggested otherwise (Table 1). Analysis of the data obtained showed that 30.3% of the studies used alloxan while 57.9% made used of STZ as a diabetogenic agent, and others which used glucose, fructose and genetic diabetic mice constituted the remaining 11.8% (Table 1).

2. Alloxan-induced diabetes

Alloxan-induced diabetes is a form of insulin-dependent diabetes mellitus that occurs as a result of alloxan administration or injection to animals [78,79]. It has been successfully induced in a variety of animal species; rabbits, mice, rats, monkeys, cats and dogs [80,81]. Alloxan has been administered in single or multiple doses, through different routes (intraperitoneal, intravenous and subcutaneous); with single intraperitoneal administration apparently the most employed mode. The dosage of the drug also varies across studies, ranging between 90 and 200 mg/kg of body weight (BW), with 150 mg/kg BW being the most frequently used dosage. Animal species, route of administration and nutritional status have been considered to play a role in determining the dose of alloxan appropriate for induction of diabetes [2]. However, single intraperitoneal administration of the drug at 170–200 mg/kg BW appears to be most effective [2].

Alloxan was first isolated by Brugnatelli in 1818 and initially described by Frederick Wohler and Justin Liebig in 1838 [83]. Alloxan causes diabetes by a mechanism which basically involves partial degradation of the beta (β) cells of pancreatic islets and subsequent compromise in the quality and quantity of insulin produced by these cells. Its use as a diabetogenic drug in experimental animals was first reported by Dunn and McLetchie in their study in which they successfully induced diabetes in experimental rabbits [78]. Thereafter, several authors have used alloxan-induced diabetes model as a "study tool" to elucidate the pathophysiology of the disease and much more as a "search engine" for antidiabetic compounds with better therapeutic characteristics.

The model employs two distinct pathological effects which include selective inhibition of glucose-stimulated insulin secretion, and induced formation of reactive oxygen species (ROS) which promotes selective necrosis of beta cells of the pancreas. Both effects collectively result in a pathophysiological state of insulin-dependent diabetes or type 1-like diabetes mellitus in cells [78,84]. The former is associated with specific inhibition of a pancreatic glucose sensor enzyme, glucokinase by alloxan whereas the latter is rather connected with the redox cycling ability of alloxan which results in ROS generation. More importantly, both effects have been linked to the chemical properties of alloxan as well as its structure.

2.1. *Chemical features of alloxan and their contribution to its diabetogenicity*

The diabetogenicity of alloxan is underlined by its selective cellular uptake by beta cells of the pancreas and consequent

Table 1 – Randomly selected experimental diabetic studies conducted within the last two decades (1995–2016).

Authors	Diabetogenic agent used	Authors	Diabetogenic agent used	Authors	Diabetogenic agent used
Kameswararao et al. [3]	Alloxan	Yadav et al. [28]	Fructose	Ighodaro et al. [53]	Alloxan
Pari and Saravanan [4]	Alloxan	Al-Azzawie and Alhamdani [29]	Alloxan	Nyomaan et al. [54]	STZ
Eidi et al. [5]	STZ	Verspoh et al. [30]	Glucose	Petchi et al. [55]	STZ
Maiti et al. [6]	STZ	Jaiswal et al. [31]	STZ	Akaladi et al. [56]	STZ
Gupta et al. [7]	STZ	Sunil et al. [32]	STZ	Olatunji et al. [57]	Fructose
Bagri et al. [8]	STZ	Jelodar et al. [33]	Alloxan	Daud et al. [58]	STZ
Tabuchi et al. [9]	STZ	Ighodaro et al. [34]	STZ	Cao et al. [59]	STZ
Ragavan and Krishnakumari [10]	Alloxan	Asgary et al. [35]	Alloxan	Saravanan et al. [60]	STZ
Dewanjee et al. [11]	Alloxan	Kumar et al. [36]	Alloxan	Hakkim et al. [61]	Alloxan
Yang et al. [12]	Alloxan	Venkatesh et al. [37]	Alloxan	Lee et al. [62]	STZ
Jala et al. [13]	Fructose	Paril et al. [38]	Alloxan	Poudyal et al. [63]	CHO/High fat
Jemai et al. [14]	Alloxan	Shanmugasundaram et al. [39]	Alloxan	Dzeufiet et al. [64]	STZ
Sridhar et al. [15]	STZ	Nugroho et al. [40]	Fructose	Anathan et al. [65]	Alloxan
Pandit et al. [16]	STZ	Jelastin et al. [41]	Alloxan	Miura et al. [66]	KK mice
Papato et al. [17]	STZ	Satheesh and Paril [42]	Alloxan	Oliveira et al. [67]	STZ
Zhang and Tan [18]	STZ	Wainstein et al. [43]	STZ	Singh et al. [68]	STZ
Sarkhail et al. [19]	STZ	Moon [44]	STZ	Bnouham et al. [69]	STZ
Habibuddin et al. [20]	STZ	Chung et al. [45]	STZ	Surana et al. [70]	Alloxan
Liu et al. [21]	STZ	Ahangarpour et al. [46]	Fructose	Anwar et al. [71]	STZ
Abdelmoaty et al. [22]	STZ	Prince et al. [47]	Alloxan	Ju et al. [72]	STZ
Oku et al. [23]	STZ	Abedinzade et al. [48]	STZ	Gandhi et al. [73]	STZ
Orhan et al. [24]	STZ	Kook et al. [49]	STZ	Shirwaikar et al. [74]	STZ
Ezquer et al. [25]	STZ	Kumar et al. [50]	STZ	Chen et al. [75]	STZ
Zhao et al. [26]	STZ	Shajeela et al. [51]	Alloxan	Djomeni et al. [76]	STZ
Singh et al. [27]	STZ	Sowmia and Kokilavani [52]	Alloxan	Veerapur et al. [77]	STZ

STZ, streptozotocin.

accumulation in these cells [85]. The chemical properties of alloxan and how they contribute to its toxicity or diabetogenicity are shown below.

2.1.1. *Alloxan shares semblance with glucose in molecular shape and hydrophilicity*

Alloxan shares huge structural (molecular shape) similarity with glucose [86]. It is a β-cell toxic glucose analog with hydrophilic characteristic (with a partition coefficient of −1.8) and exists as alloxan monohydrate in aqueous solutions [1]. Glucose is a hydrophilic molecule and hence, incapable of crossing the lipid bilayer of the plasma membrane on its own into the cytosol. Rather it is transported via a facilitated diffusion transport mechanism involving a transport protein known as glucose transporter 2 (GLUT2) which is located in plasma membranes of cells. In the same manner, due to structural similarity of alloxan to glucose, its movement across the plasma membrane into the cytosol of the beta cells is also carried out by GLUT2 [1,87].

Interestingly, alloxan does not in any way inhibit the activity of GLUT2 and this attribute significantly enhances its uptake by beta cells, resulting in its selective bio-accumulation and toxicity in these cells [88,89]. This view is substantiated by the fact that alloxan has been reported to be non-toxic to insulin-producing cells which lack or are deficient in the GLUT 2. Secondly, N-substitution with the alkyl group in alloxan produces alloxan derivatives with lipophilic characteristic and these compounds have been noted to easily transit the lipid bilayer of the plasma membrane without the assistance of glucose transporter 2, GLUT2 [90]. Consequently, they can permeate all cell types including those which do not express GLUT2 and cause systemic toxicity rather than diabetogenicity [91]. It is important to note that the mechanism of glucose uptake in humans is contrastingly different from that of animals (rodents) and this probably accounts for why alloxan, even at high concentrations are non-toxic to humans.

2.1.2. *Alloxan is a weak acid*

Alloxan is a weak acid, a barbituric acid derivative (5-ketobarbituric acid) and hence, readily attacks thiol reagents or the sulfhydryl group (–SH) present in proteins. The selective inhibition of glucose-stimulated insulin secretion has been described as the major pathological effect of alloxan [1,85] and is directly linked with the ability of alloxan to oxidized or attack the thiol group present in glucokinase, a glucose phosphorylating enzyme which plays a key role as glucose sensor in the pancreas and liver.

2.1.3. *The chemical structure of alloxan has a 5-carbonyl group*

The chemical structure of alloxan (Fig. 1) has a 5-carbonyl group which is hyper reactive with thiol groups, and this is indicative of a structure-function relationship in alloxan toxicity or diabetogenicity. Glucokinase has two thiol groups (–SH) in its binding site which makes it exceptionally susceptible to oxidation by alloxan [92]. The binding of alloxan to glucokinase results in the formation of a disulphide bond and consequent inactivation of the enzyme. This phenomenon occurs as fast as within the first minute of exposure of the enzyme to alloxan and accounts for the selective inhibition of

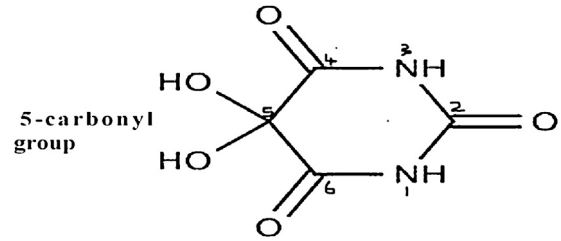

Fig. 1 – Chemical structure of alloxan.

glucose-stimulated insulin secretion usually observed within minutes of alloxan injection [86]. Although, alloxan can inhibit the activities of several other functionally important thiol-enzymes such as phosphofructokinase [93], aconitase [92], hexokinase [94] and Ca^{2+}/calmodulin-dependent protein kinase [95] but glucokinase is the most susceptible thiol enzyme to alloxan attack in the beta cells [96,97]. The inhibitory action of alloxan on glucokinase hinders glucose oxidation, and by extension the formation of adenosine triphosphate (ATP) In turn, lack of ATP suppresses the signal generating metabolic flux necessary for glucose-stimulated insulin secretion [98]. The same mechanism may likely be responsible for the inhibitory action of alloxan on insulin biosynthesis [99].

2.1.4. *Alloxan is a very unstable compound*

In addition, alloxan is a very unstable compound, a property that enables it to readily undergo redox cycling. In the presence of intracellular thiols especially glutathione (GSH), alloxan undergoes a persisting continuous cyclic reaction to generate reactive oxygen species (ROS) such as superoxide radical anion ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$) via the auto-oxidation of its reduction product, dialuric acid. The process involves the reduction of alloxan to dialuric acid and re-oxidization of dialuric acid to alloxan [100]. Re-oxidation of alloxan to dialuric acid causes a release of alloxan radical that in the presence of oxygen generates $O_2^{\bullet-}$ (Fig. 2). $O_2^{\bullet-}$ is usually dismutated to a relatively harmless hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), an antioxidant enzyme present in virtually in all tissues (Fig. 2). Catalase, another antioxidant enzyme is required to prevent the accumulation of H_2O_2 and its consequent conversion to hydroxyl radical, by quick degradation of the compound to water and molecular oxygen. However, catalase activity is very low in the pancreas [101] and as a result H_2O_2 accumulates, leading to its conversion to highly reactive hydroxyl radical through Fenton reaction (Fig. 2). Hydroxyl radical is apparently the most dangerous radical in the cell and considered to be the principally culprit in beta cell toxicity and alloxan diabetogenicity. Damage of pancreatic beta cells by ROS has been linked to fragmentation of DNA of these cells, leading to the stimulation of poly ADP-ribose polymerase 1, an enzyme that plays an important role in DNA repair process [102].

Naturally, compounds with sulfhydryl group (GSH, cysteine and dithiothreitol) should protect glucokinase against alloxan inhibition by a reductive process, but they have to continuously maintain the reduction product, dialuric acid in its reduced form in order to effectively protect the enzyme

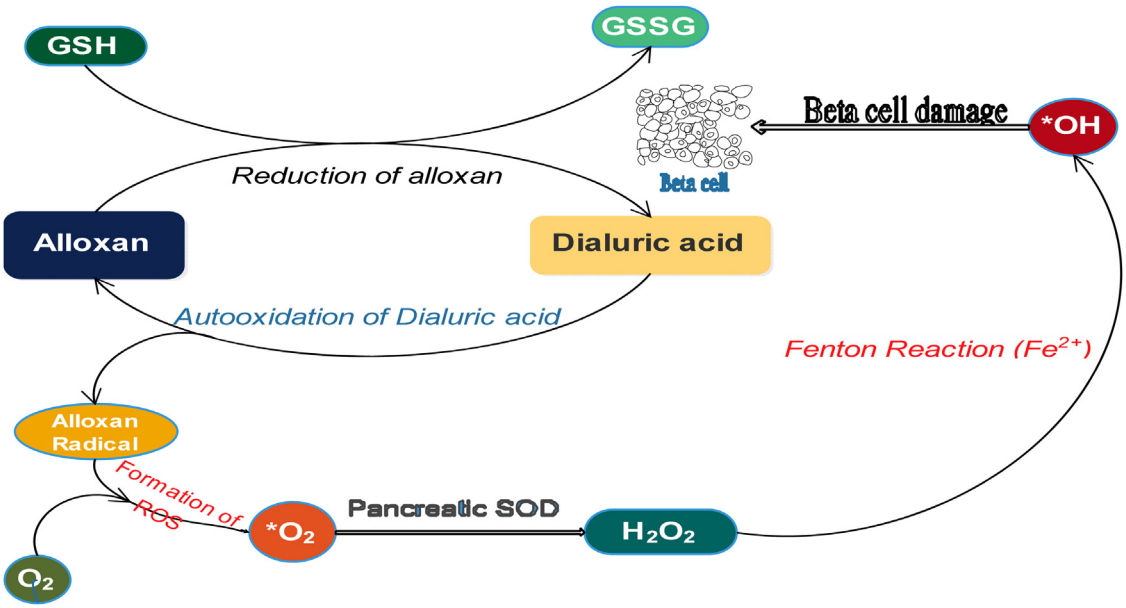

Fig. 2 – Formation of ROS through redox cycling of alloxan.

[92,103]. Unfortunately, this is not the case, as the thiols are often exhausted by the persisting continuous cyclic reaction of alloxan. Winterbourn and Munday [104] informed that the amount of reduced GSH available in a cell for redox cycling diminishes gradually and thus fosters a lower pro-oxidative ratio between alloxan and GSH, rather than a higher antioxidative ratio. This explains why co administration of thiols such as GSH or cysteine with alloxan tends to ameliorate the toxic and diabetogenic effects of alloxan as reported by a number of other studies [105–107].

2.2. *Alloxan-induced diabetes is characterized by multiphasic blood glucose response*

Alloxan induces a multiphasic blood glucose response when injected into to experimental animals as noted in our recent study in which we examined the time course effects of alloxan in Wistar rats. Within 36 h post alloxan administration, several phases of glucose response were observed in the animals administered 170 and 200 mg/kg BW alloxan (Fig. 3). The observation is similar to previous reports on blood glucose behavior or response to alloxan [1,2,85,108]. Some of these authors also noted that changes in blood glucose concentration are accompanied by corresponding inverse changes in the plasma insulin concentration.

Lenzen [1] postulated that blood glucose multiphasic response to alloxan injection begins in the first few minutes with a transient hypoglycemic phase that lasts maximally for 30 min. This event has been adduced to a transient hyperinsulinemia that is probably due to a momentary increase in ATP level resulting from the temporary effects of alloxan inhibition of glucokinase.

The second phase that usually takes place 1 h post alloxan administration is characterized by upsurge in blood glucose concentration and concomitant decrease in plasma insulin concentration. This is the first hyperglycemic effect of alloxan and it lasts for a period of 2–4 h. In our study, alloxan induced diabetic hyperglycemia (blood glucose ≥200 mg/dL or 11.1 mmol/L) in rats 1 h post its administration. Previous authors have also noted the immediate diabetogenicity of alloxan following its administration to experimental animals. Lenzen [1] in his study titled "Alloxan and streptozotocin diabetes" informed that notable hyperglycemia commenced in experimental rats 1 h after alloxan injection. Similar reports that alloxan administration to rats causes immediate hyperglycemia which reaches its peak within two or three hours' had been earlier communicated by Goldner and Gomori [80]. Inhibition of insulin secretion from the pancreatic beta cells due to ROS attack accounts for this phase of alloxan diabetogenicity [109]. According to Szkudelski [85], alloxan is a hydrophilic and unstable substance with a half-life of 1.5 min at neutral pH and 37 °C. This implies that the time for alloxan degradation (metabolism) is sufficiently short enough to allow it to reach the pancreas very fast and in deleterious amount. This also explains and buttresses the 1 h post hyperglycemic effect of alloxan.

As alloxan uptake by the insulin-secreting beta cells of the pancreas reaches its maximum, its toxicity via ROS increases, leading to induced rupture of the secretory granules and cell membrane of the beta cells [1,85,97,110,111]. The attendant effect of this burst up is flooding of the circulation with insulin, a pathophysiological condition that results in a severe transitional hypoglycemic phase which is observable a couple of hours after alloxan injection. The hypoglycemic phase in alloxan diabetogenicity has also been associated with the ability of alloxan to cause significant influx of free Ca^{2+} into the cytosol of pancreatic islet beta cells, thereby compromising the intracellular calcium homeostasis [112]. The process involves the depolarization of the pancreatic beta cells, which facilitates further calcium entry into pancreatic cells via voltage dependent calcium channels. High intracellular level of Ca^{2+} has been noted to contribute significantly to super high level of insulin release [85].

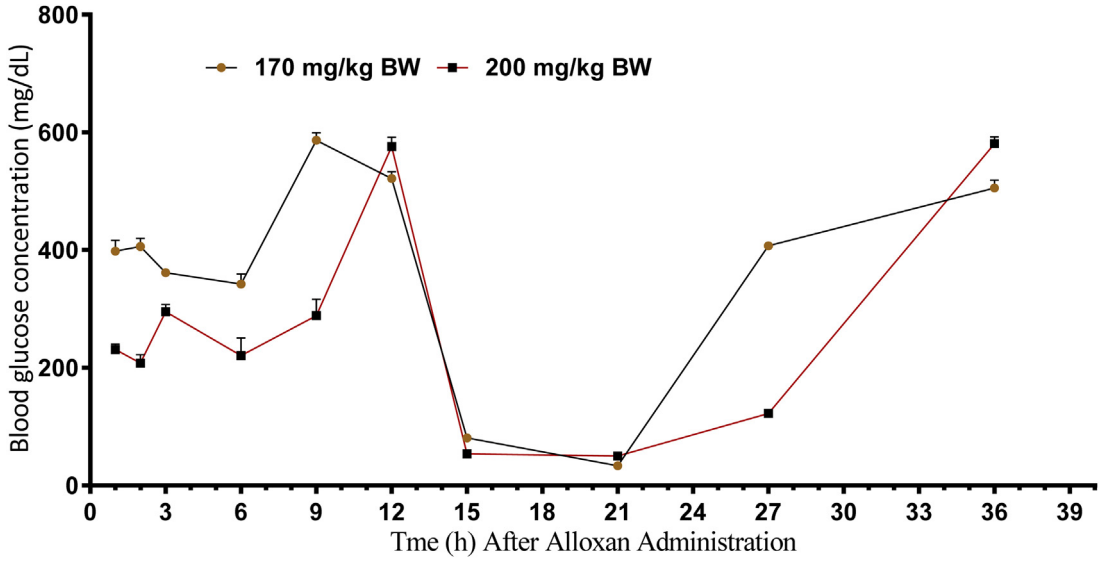

Fig. 3 – Time course effect of alloxan on blood glucose level of rat.

Hypoglycemia is characteristic of experimental diabetes and the phase has been noted to last for at least 3 h or more [1,113,114] and is largely responsible for the mortality associated with alloxan-induced diabetes. This view is consistent with the opinion of Goldner and Gomori [80], who hinted that alloxan-induced hyperglycemia in rats is followed by a severe and fatal hypoglycemia, which after a duration of several hours yields to a final hyperglycemia, last phase of alloxan induced diabetes.

The last phase of the blood glucose response to alloxan administration is touted to be a permanent diabetic hyperglycemic phase that takes place between 24 and 48 h after alloxan administration. Supposedly, there is complete degranulation and loss of structural integrity of the beta cells during this phase [1,113]. The incidence in this phase indicates that other cell types of the pancreas are spared of alloxan toxicity, substantiating the theory of selective uptake of alloxan by the insulin-producing cells (pancreatic beta cells) [115,116].

2.3. *Protective effects of glucose against alloxan toxicity and diabetogenicity*

It is a popular postulation that blood glucose protects the pancreatic islet beta cells against the toxicity and diabetogenicity of alloxan [98]. There are a couple of reasons to believe that this is possible. Principally, the mechanism by which alloxan elicits its toxicity and diabetogenicity provides that possibility. As already stated, the toxic and diabetogenic effects of alloxan are underlined by its selective inhibition of glucose-stimulated insulin secretion via inactivation of glucokinase and selective necrosis of the beta cells via induced ROS. These two processes are sequel to alloxan uptake by beta cells and subsequent accumulation in these cells.

Alloxan uptake by beta cells is facilitated by GLUT2. Since both glucose and alloxan due to similarity in molecular shape competes for the same transport protein, it therefore implies that high level of glucose in circulation will automatically lower the chance of alloxan binding to GLUT2. Even at a relatively equal concentration of both molecules, GLUT2 has a stronger affinity for glucose than alloxan and thus favors the binding of glucose compared with alloxan [90,116–118].

This effect will consequently minimize alloxan uptake and invariably affects its ability to induce diabetes. It has also been suggested that glucose through the pentose phosphate pathway has the ability to supply reduced nicotinamide adenine dinucleotide phosphate (NADPH) and reduced nicotinamide adenine dinucleotide (NADH) which are capable of recycling GSH, i.e. keeps it in its reduced form [1]. This in itself will effectively attenuate the ability of alloxan to generate reactive radicals through redox cycling. More so, high level of blood glucose will protect glucokinase and prevent its exposure to alloxan attack. This happens in such a way that once glucokinase is bound by glucose, the sulfhydryl groups in its glucose-binding site is no more available for alloxan attack [98]. The protection offered by glucose explains why fed animals are less sensitive to alloxan induced diabetes compared with fasted animals with relatively lower blood glucose level [119]. In the same manner, animals fed on fat diet have been observed to be more susceptible to alloxan diabetogenicity relative to those fed on high carbohydrate and protein prior to alloxan injection.

2.4. *Limitations of alloxan-induced diabetes*

The effectiveness of alloxan for induction of experimental diabetes has been queried by a number of investigators. This is rightly so as noticeable limitations have been associated with the use of alloxan as a diabetogenic agent. Jain and Arya [120] highlighted several anomalies and inconsistencies in alloxan-induced diabetes model, and we are of the opinion that the concerns raised by these authors should be given some level of consideration and attention.

Instability and auto-reversibility of alloxan-induced hyperglycemia is particularly of utmost concern. Alloxan when administered causes multiphasic glucose response characterized by inconsistent increase and decrease in blood glucose

concentration [1,2,108]. In other words, the hyperglycemia induced by alloxan is not sufficiently stable for proper evaluation of the antidiabetic or hypoglycemic potential of test compounds. Even in few cases where apparent stability is achieved, the duration of such stable hyperglycemia is on the average less than a month and this period is not adequate for proper evaluation of a test drug. This often leads to illusive conclusion on the antidiabetic relevance of the test compound. According to Misra and Aiman [108], a wide range of fluctuations in the blood glucose level and auto reversal from confirmed diabetic hyperglycemia to the non-diabetic range is a major setback as regards alloxan-induced diabetes model. Another problem with alloxan is that its diabetogenic and toxic effects on animals vary widely, even among those belonging to the same species. Such inconsistent effect makes the drug an unreliable model for affirming the antidiabetic potency of test compounds, a view shared by previous authors [108,120].

Moreover, alloxan does not exactly induce the human type 2 diabetes mellitus [121] which accounts for about 90–95% of all diabetic cases. In support of this, Jain and Arya [120] drew our attention to a couple of test compounds reported to have exhibited notable antidiabetic activities against alloxan-induced diabetes but were found to be ineffective against human diabetes.

Alloxan has been noted to stimulate a type 1 form of diabetes when used in animals. This form of diabetes is often associated with high level of ketoacidosis that arguably is partly responsible for the high animal mortality rate (30–60%) [120] usually observed with use of alloxan as a diabetogenic agent. Besides, the mechanism of alloxan diabetogenicity encloses a chronic measure of toxicity involving free radical generation, particularly ($^{\bullet}OH$). No doubt, this play a bigger role in the mortality of experimental animals exposed to alloxan. Mortality from diabetes has been adduced to either initial hypoglycemic shock or emergence of diabetic complications or direct kidney tubular cell toxicity [85].

The practice of placing alloxan-treated animals on 5–10% glucose solution in a bid to prevent hypoglycemic shock is often observed but this intervention appears not to be significantly helpful, and thus the problem of mortality persists. High mortality rate is a major drawback in the use of alloxan diabetic model. First, it increases the financial burden of the study as several animals more than required have to be used in attempt to carry the study to a meaningful end. Secondly, it does not allow for proper evaluation of the antidiabetic potential of the investigated compound or test drug.

2.5. *Comparing alloxan with streptozotocin as diabetogenic agents*

STZ has notable advantages over alloxan as chemical agents for induction of experimental diabetes, thus, is often preferred to the latter (alloxan). For instance, STZ has longer half-life (15 min against 1.5 min of alloxan) [122]. This makes it more stable in solution before and after injection into animals. STZ-induced hyperglycemia is relatively more stable and for a longer duration (as much as three months compared to alloxan-induced hyperglycemia that can only be sustained for less than a month). Moreover, the mechanism of STZ diabetogenicity is less associated with cellular toxicity, hence, lesser animal mortality. Alloxan on the contrary, induces diabetes by a mechanism characterized by incidences of ketosis, ROS toxicity, and high mortality rate which is particularly a major setback in experimental diabetes studies [85]. One reason for this is that STZ is more selective to islet beta cells than alloxan which causes severe damage to other cell types which express GLUT2 (systemic toxicity).

More so, STZ-induce diabetes is associated with well characterized diabetic complications unlike alloxan-induced diabetes [1]. In addition, compared to alloxan, STZ diabetogenicity is not severely interfered with by blood glucose level. Overall, STZ diabetogenicity is more effective and with lesser variation with animal species.

2.6. *Suggestions to improve the use of alloxan as a diabetogenic drug*

1. Alloxan is very unstable, and with a half-life of about 1.5 min, it could easily disintegrate when left to stand in aqueous solutions. Therefore, when used as a diabetogenic agent, it should be freshly prepared. In a case where the animals to be injected are quite many, it is advisable that the appropriate amount of alloxan for a specific number of animals (i.e. 5) is measured in replicates for different batches of animals. This means that alloxan for a batch of animals ($n = 5$) is dissolved in freshly prepared 0.9% saline just before the commencement of administration. This practice improves alloxan diabetogenicity. On the contrary, when all the animals in a large group is injected from the same alloxan preparation, there is a possibility that the last set of animals administered the drug may not receive sufficient amount of the active drug due to disintegration. According to Lenzen and Munday [123], alloxan when left to stand in aqueous solutions is readily converted to non-diabetogenic alloxanic acid due to spontaneous decomposition.
2. Poor diabetogenicity and easy auto-reversal of alloxan-induced hyperglycemia is very common with intraperitoneal doses of 150 mg/kg and below [85,124]. In the use of alloxan, higher dose between 170 and 200 mg/kg BW have been noted to be more effective.
3. Very young animals have been observed to be highly resistant or less susceptible to the diabetogenic effect of alloxan [120,125]. Older animals should be preferably used in diabetic studies involving the use of alloxan. Antioxidant defense system has been reported to decrease with age; this may be responsible for this difference in age–dependent response to alloxan.
4. Fed animals due to the effect of blood glucose are less susceptible to alloxan toxicity and diabetogenicity [84,85]. Animals should therefore be fasted for at least 12 h prior to alloxan injection. Fasted animals have relatively low blood glucose level. This physiological condition enhances alloxan uptake by the islet beta cells and consequently improve alloxan diabetogenicity.
5. Exogenous GSH has been reported to protect well against alloxan toxicity which is often connected with animal mortality [1]. Probably, co-administration of very low

concentration of GSH and higher dose of alloxan (170–200 mg/kg) should be considered for improved diabetogenicity of alloxan.

6. The route and speed of administration have been reported to affect the diabetogenicity of alloxan, with fast or rapid intravenous administration preferred to slow intravenous and intraperitoneal (I.P.) administration. But higher rate of mortality have also been associated with rapid intravenous injection [108]. In alloxan diabetes studies, intraperitoneal injection is commonly used. Perhaps, increasing the speed of intraperitoneal administration may improve alloxan diabetogenicity.

Conflict of interest

The authors state no conflict of interest.

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