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Diabetogenic potential of Alloxan in Albino Mice

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Abstract: *Alloxan, a derivative of urea is known to be a carcinogen and cytotoxic glucose analog. It is also known to be one of the most common diabetogenic agents to assay the antidiabetic efficacy of plant extracts and pure compounds in a number of diabetic animal models.*

In the present investigation the diabetogenic efficacy of different concentrations of alloxan has been assayed on albino mice. The results revealed that alloxan induced a multiphasic plasma glucose response when injected intraperitoneally in albino mice. 50 mg/kg BW and 100 mg/kg BW of alloxan did not cause a rise in plasma glucose level up to 50 hours. After 50 hours of exposure 50 mg/kg/W and 100 mg/kg BW cause a rise in the plasma glucose level to 115.2 mg/dL and 120.0 mg/dL respectively that was within the normal reference range. The hyperglycemic activity of albino mice increased on increasing the concentration of alloxan and time of exposure. At 150 mg/kg BW of alloxan the hyperglycemic activity was noticed only after35 hours of exposure (207.0 mg/dL of blood). At this concentration of alloxan the plasma glucose level increased to 265.0 mg/dL of blood after 50 hours of exposure. The diabetogenic activity of alloxan at 200 mg/kgBW and 250 mg/kg BW was noticed only after 10 hours exposure. After 50 hours of exposure these two concentrations of alloxan caused significant increase in plasma glucose level to 360.0 and 525.0 mg/dL of blood respectively. Alloxan at the concentration of 150 mg/kg BW could be considered as mild diabetogenic, but200 mg/kg BW and 250 mg/kg BW as potent inducer of diabetes mellitus.

Key Words: Alloxan, Albino Mice, Diabetogenic agent

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I. Introduction

Alloxan (5, 5-dihydroxyl pyrimidine- 2, 4, 6-trione) is a derivative of urea and is known to be a carcinogen and cytotoxic glucose analog (Fig-1) (Lenzen, 2008) [1].The molecular formula and molecular mass of alloxan are $C_4H_2N_2O_4$ and 142.06 respectively. It is known to be one of the most common diabetogenic agents to assay the antidiabetic efficacy of plant extracts and pure compounds in a number of diabetic animal models viz. rabbits, mice, rats, monkeys, cats and dogs (Goldner and Gomori, 1945; Cruz *et al*., 1961) [2, 3].

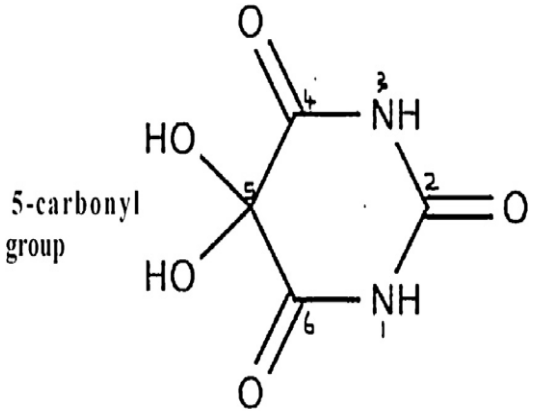

Figure-1: Chemical structure of alloxan

Alloxan induces insulin-dependent diabetes mellitus (Type-1) (Dunn and McLetchie, 1943; Gomori and Goldner, 1945) [4, 5]. Alloxan can be administered through different routes viz. intraperitoneal, intravenous and subcutaneous with single or multiple doses. The genetic strains, route of administration and nutritional status of animal species play a role in determining the appropriate dose of alloxan for induction of diabetes (Federiuk *et al*., 2004) [6]. However, single intraperitoneal administration of the drug at 170–200 mg/kg BW is shown to be the most effective dose (Federiuk *et al*., 2004) [6].

Alloxan induces diabetes mellitus in experimental animals by a mechanism which basically involves partial degradation of the beta (β) cells of islet of langerhans in pancreas and subsequent compromise in the quality and quantity of insulin produced by these cells. The alloxan induces two distinct pathological effects. First, selective inhibition of glucose-stimulated insulin secretion, and second, the formation of reactive oxygen species (ROS) which promotes selective necrosis of beta cells of the pancreas. Both these effects collectively result in a pathophysiologi cal state of insulin-dependent diabetes or Type 1like diabetes mellitus in animals (Dunn and McLetchie, 1943; Jorns *et al*., 1997) [4, 7]. 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 (Fig-2).

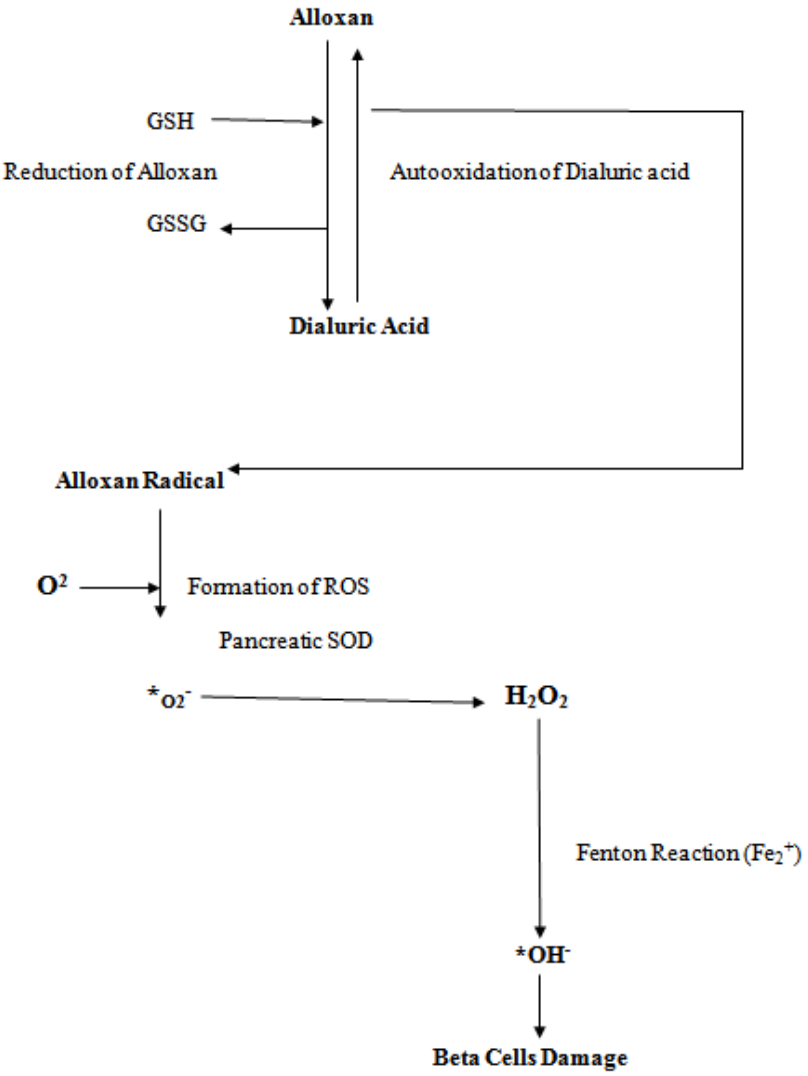

Fig- 2: Formation of ROS through redox cycling of alloxan.

Structurally alloxan resembles glucose in terms of molecular shape (Weaver *et al*., 1979) [8] and is toxic to β-cells and exists as alloxan monohydrate in aqueous solutions (Lenzen, 2008) [1]. Glucose 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. Its movement across the plasma membrane into the cytosol of the beta cells is also carried out by GLUT2 (Lenzen, 2008; Gorus *et al*., 1982) [1, 9]. It is interesting that alloxan does not 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 (Malaisse *et al*., 2001; Elsner *et al*., 2002) [10, 11]. Alloxan has been known to be non-toxic to insulin-producing cells (β-cells) which lack or are deficient in the GLUT 2. The N-substitution with the alkyl group in alloxan produces alloxan derivatives with lipophilic characteristic and these compounds easily transported to the lipid bilayer of the plasma membrane without the assistance of glucose transporter 2, GLUT2 (Elsner *et al*., 2002) [11]. Consequently, they can permeate all cell types including those which do not express GLUT2 and cause systemic toxicity rather than diabetogenicity (Bruckmann *et al*., 1947) [12].

Alloxan is a weak acid which is a derivative of barbituric acid (5- ketobarbituric acid). It readily attacks thiol (-SH) group of proteins. The selective inhibition of glucose-stimulated insulin secretion is the major pathological effect of alloxan (Lenzen, 2008; Szkudelski, 2001) [1, 13] and is directly linked with the ability of alloxan to oxidize the thiol group present in glucokinase which plays a key role as glucose sensor in the pancreas and liver. Alloxan has five carbonyl group which is hyper reactive with thiol group. The glucokinase has two thiol groups which are susceptible to oxidation by carbonyl group of alloxan (Lenzen and Mirzai-Petri, 1992) [14]]. Binding of alloxan to glucokinase results in the formation of a disulphide bond and consequent inactivation of the enzyme. This phenomenon occurs within one minute of exposure of the enzyme to alloxan. The selective inhibition of glucose-stimulated insulin secretion usually observed within a few minutes of alloxan injection (Weaver *et al*., 1979) [8]. Although, alloxan can inhibit the activities of several other functionally important thiol-enzymes such as phosphofructokinase (Garland *et al*., 1963) [15], aconitase (Lenzen and Mirzai-Petri, 1992) [14], hexokinase (Lenzen *et al*., 1990) [16] and Ca2+/calmodulin-dependent protein kinase (Colla *et al*., 1983) [17] but glucokinase is the most susceptible thiol enzyme to alloxan attack in the beta cells (Tiedge *et al*., 2000; Borg *et al*., 1979) [18, 19]. The inhibitory action of alloxan on glucokinase hinders glucose oxidation and the formation of adenosine triphosphate (ATP). The lack of ATP, in turn suppresses the signal generating metabolic flux necessary for glucose-stimulated insulin secretion (Lenzen and Panten, 1988) [20]. The same mechanism is also responsible for the inhibitory action of alloxan on insulin biosynthesis (Niki *et al*., 1976) [21].

In the presence of intracellular thiols of Glutathione (GSH), alloxan undergoes a persisting continuous cyclic reaction which generates reactive oxygen species (ROS) such as superoxide radical anion (O_2^{*-}) and hydroxyl radical ($^{*}OH$) via the autooxidation of its reduction products, dialuric acid. The process involves the reduction of alloxan to dialuric acid and re-oxidation of dialuric acid to alloxan (Munday, 1988) [22].

Re-oxidation of alloxan to dialuric acid causes a release of alloxan radical that in the presence of oxygen generates $O2^{*-}$ radical. $O2^{*-}$ radical is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). Catalase 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 (Burkart *et al.*, 1993) [23] and as a result H_2O_2 accumulates, leading to its conversion to highly reactive hydroxyl radical through Fenton reaction. Hydroxyl radical is apparently the most dangerous radical in the cell and considered to be the principally culprit in beta cell toxicity and alloxan induced 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 (Brownlee, 2005) [24]. The compounds with SH (sulfahydril) group protect glucokinase against alloxan inhibition by a reductive process, but they have to continuously maintain the reduction product, dialuric acid in its reduced state to protect the enzyme effectively (Lenzen and Mirzai-Petri, 1992; Elsner et *a*l., 2006) [14, 25]. Winterbourn and Munday (1989) [26] suggested 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 compounds with thiol group viz. GSH or cysteine with alloxan tends to ameliorate the toxic and diabetogenic effects of alloxan (Lazarow, 1946; Lazarow *et al*., 1948; Sen and Bhattacharya, 1952) [27, 28, 29].

Alloxan-induced diabetogenic animals have been developed by several workers viz. Kameswar rao et al., (2003) [30], Pari and Saravanan (2002) [31], Ragavan and Krishnakumari (2006) [32], Dewanjee et al., (2008) [33], Yang et al., (2000) [34], Jemai *et al*., (2009) [35], Ighodaro *et al*., (2012) [36], Hakkin *et al*., (2007) [37], Anathan *et al*., (2003) [38], Surana *et al*., (2008) [39], Al-Azzawie and Alhamdani (2006) [40], Asgary *et al*., (2008) [41], Jeloder *et al*., (2005) [42], Kumar *et al*., (2009) [43], Venkatesh *et al*., (2010) [44], Paril *et al*., (2004) [45], Shanmugasundaran *et al*., (2011) [46], Prince *et al*., (2004) [47], Shajeela *et al*., (2012) [48], Sowmia and Kokilabani (2007) [49] etc. Alloxan-induced diabetogenic mice of albino genetic background have not been developed for the experimental assay of hypoglycemic efficacy of plant extracts till today. Hence the present investigation was undertaken. The aim of present study is to develop diabetogenic albino mice using different concentrations of alloxan so that the hypoglycemic efficacy of plant extracts can successfully be performed.

II. Materials And Methods

All chemicals and reagents viz. alloxan, Sodium chloride, citric acid, sodium citrate etc. were purchased from Merck, India and were of analytical grade. Blood glucose diagnostic kit was purchased from Span diagnostic Limited, Surat, India.

Significant insights into the etiology of diabetes mellitus in human have been gained from the study of animal models. The albino mouse is an excellent model for study of human diabetes. Therefore, mice used in this study were of the albino genetic background. Adult albino mice weighing around 20-25 gram with 6.5 ± 0.5 cm length were procured from Mahabeer Cancer Sansthan, Patna. The mice were housed in shoe-box type cages under good hygienic conditions in the departmental animal house during experimental period. The mice were allowed to acclimatize for 15 days in an environmentally controlled room under standard environmental conditions (21±2°C; 55±5% humidity, 12 hr Light: Dark cycle). The mice were fed with diet consisted of following ingredients:

Wheat grains:	1 Kg
Choker wheat:	250 gm
Gram grains:	250 gm
Maize grains:	250 gm
Soybean grains:	250 gm
Sun drop oil:	50 gm
Milk powder:	2 table spoon
Jaggery (Gudd):	50 gm

The diet consisted of following nutrients:

Carbohydrate:	48.3%
Crude protein:	23.5%
Crude fat:	5.9%
Crude ash:	5.9%
Crude fiber:	3.9%

Pellet of diet of about 5 gram was prepared and given daily to each cage at the rate of one pellet per mice.

Preparation of Stock Solution of Alloxan

The citrate buffer was prepared as follows:

A: 0.1 M solution of citric acid [$C_6H_8O_7.H_2O$ (Mol. Wt. = 210.14)] (21.01 gm in 1000 ml).

B: 0.1 M solution of sodium citrate [$C_6H_5Na_3O_7.2H_2O$ (Mol. Wt. = 294.10)] (29.41 gm in 1000 ml).

Accordingly, 28.0 ml of A and 22.0ml of B were dissolved in 100 ml distilled water for making the citrate buffer at the pH 4.5 and always prepared fresh for immediate use.

Five different concentrations of alloxan were prepared in citrate buffer at pH 4.5 for induction of diabetes in albino mice. These were 50 mg/kg BW, 100 mg/kg BW, 150 mg/kg BW, 200 mg/kg BW and 250 mg/kg BW.

Induction of Diabetes

The albino mice model for the current study was based on intraperitoneal administration of five different doses of alloxan viz. 50 mg/kg BW, 100mg/kg BW, 150mg/kg BW, 200mg/kg BW and 250mg/kg BW separately. Initially the normal mice were kept 24 hours without food and water. The weight of normal mice was recorded. Diabetes was induced by multiple intra-peritoneal injection of freshly prepared alloxan solution in 0.05 M sodium citrate (pH 4.5) at the five different doses at the intervals of five hours. The mice were then allowed to access the respective food and water *ad libitum*. Mice with fasting blood glucose level of 200 mg/dL (7.8 mmol/L) or higher were considered as diabetic. A parallel set of control mice (non-diabetic) were injected with citrate buffer only. Plasma glucose level was recorded at every five hours up to fifty hours of alloxan administration.

All the mice were fed with common pellet of diets for one month after arrival, and then randomly divided into two groups. One group continued to receive common pellet diets and constituted the normal control group; the other was injected with five different doses of alloxan separately in order to induce diabetes mellitus. All the mice had free access to food and water. Three mice were used for each group (three for normal control group and three mice of each of the five concentrations of the drug alloxan.

Body weights were recorded weekly during the experimental period. The results were recorded after one week of alloxan administration. Blood samples were taken after 8 hrs fasting from the retro-orbital sinus vein under mild ether anesthesia and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out.

Plasma glucose was estimated on the basis of *glucose oxidase*/*peroxidase* (GOD-POD). Glucose is oxidized by *glucose oxidase* to produce gluconate and hydrogen peroxide. The hydrogen peroxide then oxidatively couples with 4-aminoantipyrine and phenol to generate quinoneimine. This coloured complex produced is proportional to the glucose concentration in the sample which was measured spectrophotometrically at 500 nm with a UV-Vis spectrometer. The reaction scheme is shown below:

$$\text{Glucose} + 1/2O_2 \longrightarrow \text{Gluconate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \longrightarrow \text{Quinoneimine} + 4H_2O$$

Data were expressed as the mean ± S.E and analyzed ANOVA (one-way analysis of variance). The results obtained have been presented in Table-1 and Figure-3.

Table-1: Plasma glucose level in mg/dL of albino mice after administration of different concentration of Alloxan at different time intervals

Plasma glucose level in reference range	Plasma glucose level in control mice	Concentration of Alloxan administered (mg/kg BW)	Exposure time intervals in hours									
70-110	72.15 ±1.02		5	10	15	20	25	30	35	40	45	50
		50	75.0 ±1.16	85.0 ±1.75	99.3 ±1.65	105.0 ±1.16	108.0 ±0.66	110.0 ±1.66	110.0 ±0.75	112.0 ±1.51	113.0 ±0.67	115.2 ±0.84
		100	76.0 ±1.12	87.0 ±1.72	95.2 ±1.15	103.5 ±1.26	109.3 ±1.28	112.0 ±1.36	116.0 ±1.25	118.0 ±1.15	118.0 ±0.76	120.0 ±1.05
		150	83.0 ±1.10*	97.0 ±1.65*	112.3 ±1.35*	140.2 ±1.16*	165.5 ±1.35*	185.1 ±1.35*	207.0 ±1.61*	218.0 ±1.65*	240.0 ±1.64*	265.0 ±1.36*
		200	160.0 ±1.06*	205.0 ±1.45*	246.1 ±1.15*	275.4 ±1.31*	300.0 ±1.25*	320.2 ±1.15*	344.0 ±1.25*	350.0 ±1.41*	358.3 ±1.26*	360.0 ±1.66*
		250	180.0 ±1.21*	220.0 ±1.25*	265.0 ±1.32*	295.5 ±1.05*	315.3 ±1.21*	376.0 ±1.41*	425.0 ±1.35*	485.2 ±1.71*	507.0 ±1.63*	525.0 ±1.25*

*Significant at $P<0.05$ as compared with control

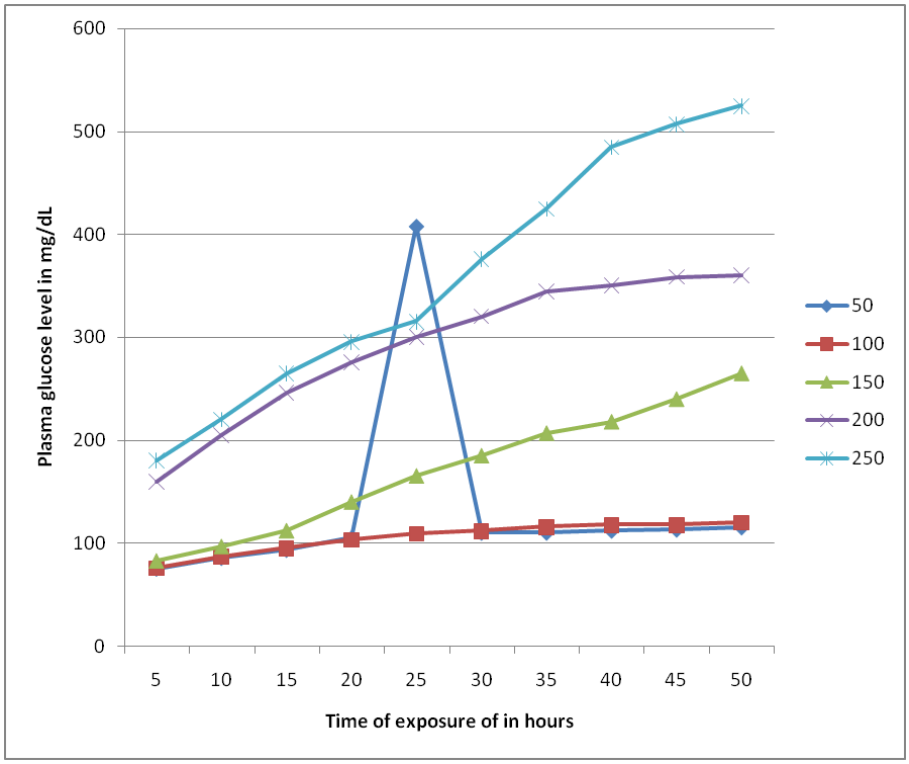

Figure-3: Plasma glucose level of albino mice after administration of five different concentrations of alloxan (50, 100, 150, 200 and 250 mg/ kg BW) at different exposure time

III. Results

From the results it is evident that alloxan induced a multiphasic plasma glucose response when injected intraperitoneally in albino mice. It was observed that 50 mg/kg BW and 100 mg/kg BW of alloxan did not cause a rise in plasma glucose level up to 50 hours of exposure. After 50 hours of exposure alloxan at the concentrations of 50 mg/kg/W and 100 mg/kg BW caused a rise in the plasma glucose level to 115.2 mg/dL and 120.0 mg/dL respectively that was within the normal reference range (Table-1; Fig-3). The hyperglycemic activity of albino mice increased on increasing the concentration of alloxan and time of exposure. At 150 mg/kg BW of alloxan concentration the hyperglycemic activity was noticed only after35 hours of exposure (207.0 mg/dL of blood). At this concentration of alloxan the plasma glucose level increased to 265.0 mg/dL of blood after 50 hours of exposure. The diabetogenic activity of alloxan at 200 mg/kgBW and 250 mg/kg BW was noticed only after 10 hours exposure. After 50 hours of exposure these two concentrations of alloxan caused significant increase in plasma glucose level to 360.0 and 525.0 mg/dL of blood respectively (Table-1; Fig-3). The 150 mg/kg BW could be considered as mild diabetogenic, but200 mg/kg BW and 250 mg/kg BW as potent inducer of diabetes mellitus.

IV. Discussion

In the present investigation it was found that within 50 hours of alloxan administration, several phases of glucose response were observed. The present findings are more or less similar to the results on plasma glucose response to alloxan in rats (Lenze, 2008; Federiuk *et al*., 2004; Szkudelski, 2001; Misra *et al*., 2012) [1, 6]. Lenzen (2008) [1] reported that the plasma glucose multiphasic response to alloxan injection begins in the first few minutes with a transient hypoglycemic phase that lasts maximally for 30 min. This is considered to be 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 is characterized by upsurge in blood glucose concentration and concomitant decrease in plasma insulin concentration. This occurs one hour after alloxan administration. This represents the first hyperglycemic effect of alloxan and it lasts for a period of 2-4 hours. In the present study, alloxan induced diabetic hyperglycemia (blood glucose ≥200 mg/dL or 11.1 mmol/ L) in albino mice occurs only after 10 administration at concentrations 200 mg/kgBW and 250 mg/kgBW. Similar results were recorded by Goldner and Gomori (1945) [2] in rats after alloxan administration. Inhibition of insulin secretion from the pancreatic beta cells due to ROS attack accounts for this phase of alloxan diabetogenicity (Kliber and Schindler, 1996) [50]. Szkudelski Szkudelski, (2001) [13] reported that alloxan is a hydrophilic and unstable substance with a half-life of 1.5 min at neutral pH and 37 8^0C. This means that the time for alloxan degradation (metabolism) is sufficiently short enough to allow it to reach the pancreas very fast and in deleterious amount.

As uptake of alloxan by the beta cells of the pancreas reaches its maximum, its toxicity via ROS increases. This leads to induced rupture of the secretary granules and cell membrane of the beta cells (Lenze, 2008; Szkudelski, 2001; Borg *et al*., 1979; Bailey, 1949; Mythili *et al*., 2004) [1, 13, 19, 51, 52] leading to flooding of the circulation with insulin. This is a pathophysiological condition that results in a severe transitional hypoglycemic phase. 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 (Park *et al*., 1995) [53]. 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 reporeted to contribute significantly to super high level of insulin release (Szkudelski, 2001) [13].

The last phase of the blood glucose response to alloxan administration is the permanent diabetic hyperglycemia that takes place 20 to 50 hours of alloxan administration. In this phase there is complete degranulation and loss of structural integrity of the beta cells of pancreatic islets of langerhans (Lenzen, 2008; Tasaka *et al*., 1988; Elsner *et al*., 2008; Bansal *et al*., 1980) [1, 54, 55, 56]. Alloxan is known to stimulate Type 1 diabetes mellitus when used in animals. This form of diabetes mellitus is associated with high level of ketoacidosis that is responsible for the high rate of animal mortality (30-60 (Jain and Arya, 2011) [57] which is observed with use of alloxan as a diabetogenic agent. Elsner et al., (2003), Misra et al., (2012) and Saravanan et al., (2010) [58, 59, 60] have also observed a more or less similar pattern as diabetogenic agent in rats.

V. Conclusion

From the present investigation it can be concluded that the alloxan is a diabetogenic agent when administered to albino mice. The diabetogenic efficacy of alloxan is concentration dependent. The 150 mg/kg BW can be considered as mild diabetogenic, but200 mg/kg BW and 250 mg/kg BW as potent inducer of diabetes mellitus.

Conflict of Interest: Authors state no conflict of interest

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References

[1]. Lenzen S (2008): The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* **51(2):**216–26
[2]. Goldner MG, Gomori G (1944): Studies on the mechanism of alloxan diabetes. Endocrinology **35 (4):**241–8.
[3]. Cruz AB, Amatuzio DS, Grande F, Hay LJ (1961): Effect of intra-arterial insulin on tissue cholesterol and fatty acids in alloxan-diabetic dogs. *Circ Res*, **9 (1):**39–43.
[4]. Dunn JS, McLetchie NG (1943): Experimental alloxan diabetes in the rat. *Lancet*, **242(6265):**384–7.
[5]. Gomori G, Goldner MG (1945): Acute nature of alloxan damage. *Proc Soc Exp Biol Med* **58 (3):2**32–3.
[6]. Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward KW (2004): Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment. *Compar Med*, **54 (3):**252–7.
[7]. Jorns A, Munday R, Tiedge M, Lenzen S (1997): Comparative toxicity of alloxan, N-alkylalloxans and ninhydrin to isolated pancreatic islets in vitro. *J Endocrinol*, **155 (2):**283–93.
[8]. Weaver DC, Barry CD, Mcdaniel ML, Marshall GR, Lacy PE (1979): Molecular requirements for recognition at a glucoreceptor for insulin release. *Molec Pharmacol*, **16 (2):**361–8.
[9]. Gorus FK, Malaisse WJ, Pipeleers DG (1982): Selective uptake of alloxan by pancreatic B-cells. *Biochem J*, **208 (2):**513–5.
[10]. Malaisse WJ, Doherty M, Ladriere LA (2001): Malaisse-Lagae FR. Pancreatic uptake of [2-14C] alloxan. *Int J Molec Med*, **7 (3):**311–5.
[11]. Elsner M, Tiedge M, Guldbakke B, Munday R, Lenzen S (2002): Importance of the GLUT2 glucose transporter for pancreatic beta cell toxicity of alloxan. *Diabetologia*, **45 (11):**1542–9.
[12]. Brückmann G, Wertheimer E (1947): Alloxan studies: the action of alloxan homologues and related compounds. *J Biol Chem*, **168 (1)**:241–56.
[13]. Szkudelski T (2001): The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*, **50 (6):**537–46.
[14]. Lenzen S, Mirzaie-Petri M (1992): Inhibition of aconitase by alloxan and the differential modes of protection of glucose, 3-O-methylglucose, and mannoheptulose. Naunyn-Schmiedeberg's *Arch Pharmacol*, **346 (5):**532–6.
[15]. Garland PB, Randle PJ, Newsholme EA (1963): Citrate as an intermediary in the inhibition of phosphofructokinase in rat heart muscle by fatty acids, ketone bodies, pyruvate, diabetes and starvation. *Nature*, **200 (4902):**169–70.
[16]. Lenzen S, Freytag S, Panten U, Flatt PR, Bailey CJ (1990): Alloxan and ninhydrin inhibition of hexokinase from pancreatic islets and tumoural insulin-secreting cells. *Basic Clin Pharmacol Toxicol*, **66 (3):**157–62.
[17]. Colca JR, Brooks CL, Landt M, McDANIEL ML (1983): Correlation of Ca2+-and calmodulin-dependent protein kinase activity with secretion of insulin from islets of Langerhans. *Biochem J*, **212 (3):**819–27.
[18]. Tiedge M, Elsner M, McClenaghan NH, Hedrich HJ, Grube D, Klempnauer J, et al. (2000): Engineering of a glucose-responsive surrogate cell for insulin replacement therapy of experimental insulin-dependent diabetes. *Hum Gene Therapy*, **11(3):**403–14.
[19]. Borg LH, Eide SJ, Andersson A, Hellerström C (1979): Effects in vitro of alloxan on the glucose metabolism of mouse pancreatic B-cells. *Biochem J*, 182(3):797–802.
[20]. Lenzen S, Panten U (1988): Signal recognition by pancreatic B-cells. *Biochem Pharmacol*, **37(3):**371–8.
[21]. Niki A, Niki H, Miwa I, Lin BJ (1976): Interaction of alloxan and anomers of D-glucose on glucose-induced insulin secretion and biosynthesis in vitro. *Diabetes*, **25(7):**574–9.
[22]. Munday R. Dialuric acid autoxidation (1988): effects of transition metals on the reaction rate and on the generation of ''active oxygen'' species. *Biochem Pharmacol*, **37(3):**409–13.
[23]. Burkart V, Koike T, Brenner HH, Imai Y, Kolb H (1993): Dihydrolipoic acid protects pancreatic islet cells from inflammatory attack. *Inflamm Res*, **38:**60–5.
[24]. Brownlee M (2005): The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*, **54(6):**1615–25.
[25]. Elsner M, Gurgul-Convey E, Lenzen S (2006): Relative importance of cellular uptake and reactive oxygen species for thetoxicity of alloxan and dialuric acid to insulin-producing cells. *Free Radic Biol Med*, **41 (5):**825–34.
[26]. Winterbourn CC, Munday R (1989): Glutathione-mediated redox cycling of alloxan: mechanisms of superoxide dismutase inhibition and of metal-catalyzed OH formation. *Biochem Pharmacol*, **38(2):**271–7.
[27]. Lazarow A (1946): Protective effect of glutathione and cysteine against alloxan diabetes in the rat. *Proc Soc Exp Biol Med*, **61(4):**441–7.
[28]. Lazarow A, Patterson JW, Levey S (1948): The mechanism of cysteine and glutathione protection against alloxan diabetes. *Science*, **108 (2803):**308–9.
[29]. Sen PB, Bhattacharya G (1952): Protection against alloxan diabetes by glucose. *Indian J Physiol*, 6:112–4.
[30]. Kameswararao B, Kesavulu MM, Apparao C (2003): Evaluation of antidiabetic effect of Momordica cymbalaria fruit in alloxan-diabetic rats. *Fitoterapia*, **74(1):**7–13.
[31]. Pari L, Saravanan G (2002): Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. *Compar Biochem Physiol C*: *Toxicol Pharmacol*, **131(1):**19–25.
[32]. Ragavan B, Krishnakumari S (2006): Antidiabetic effect of T. arjuna bark extract in alloxan induced diabetic rats. *Indian J Clin Biochem*, **21 (2):**123.
[33]. Dewanjee S, Bose S, Sahu R, Mandal S (2008): Antidiabetic effect of matured fruits of Diospyros peregrina in alloxan-induced diabetic rats. *Int J Green Pharm*, **2 (2):**95.
[34]. Yang XB, Huang ZM, Cao WB, Zheng M, Chen HY, Zhang JZ (2000): Antidiabetic effect of Oenanthe javanica flavone. *Acta Pharmacol Sin*, **21 (3):**239–42.
[35]. Jemai H, El Feki A, Sayadi S (2009): Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. *J Agric Food Chem*, **57 (19):**8798–804.
[36]. Ighodaro OM, Omole JO, Adejuwon AO, Odunaiya AA (2012): Effects of Parinari polyandra seed extract on blood glucose level and biochemical indices in Wistar Rats. *Int J Diabetes Res*, **1(4):**68–72.
[37]. Hakkim FL, Girija S, Kumar RS, Jalaludeen MD (2007): Effect of aqueous and ethanol extracts of Cassia auriculata L. flowers on diabetes using alloxan induced diabetic rats. *Int J Diabetes Metab*, January **(15):**100–6.
[38]. Ananthan R, Baskar C, NarmathaBai V, Pari L, Latha M, Ramkumar KM (2003): Antidiabetic effect of Gymnema montanum leaves: effect on lipid peroxidation induced oxidative stress in experimental diabetes. *Pharmacol Res*, **48 (6):**551–6.
[39]. Surana SJ, Gokhale SB, Jadhav RB, Sawant RL, Wadekar JB (2008): Antihyperglycemic activity of various fractions of Cassia auriculata Linn. in alloxan diabetic rats. *Indian J Pharm Sci*, **70 (2):**227.
[40]. Al-Azzawie HF, Alhamdani MS. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci 2006;78(February (12)):1371–7.
[41]. Asgary S, Parkhideh S, Solhpour A, Madani H, Mahzouni P, Rahimi P (2008): Effect of ethanolic extract of *Juglans regia* L. on blood sugar in diabetes-induced rats. *J Med Food*, 11 **(3):533**–8.

[42]. Jelodar G, Maleki M, Motadayen M, Sirus S. (2005): Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. *Indian J Med Sci*, **59 (2):**64.
[43]. Kumar V, Khanna AK, Khan MM, Singh R, Singh S, Chander R, et al. (2009): Hypoglycemic, lipid lowering and antioxidant activities in root extract of Anthocephalus indicus in alloxan induced diabetic rats. *Indian J Clin Biochem*, **24 (1):**65–9.
[44]. Venkatesh S, Reddy BM, Reddy GD, Mullangi R, Lakshman M (2010): Antihyperglycemic and hypolipidemic effects of Helicteres isora roots in alloxan-induced diabetic rats: a possible mechanism of action. *J Nat Med*, **64(3):**295–304.
[45]. Pari L, Amarnath Satheesh M (2004): Antidiabetic effect of Boerhavia diffusa: effect on serum and tissue lipids in experimental diabetes. *J Med Food*, **7(4):**472–6.
[46]. Shanmugasundaram R, Devi KV, Soris TP, Maruthupandian A, Mohan VR (2011): Antidiabetic, antihyperlipidaemic and antioxidant activity of *Senna auriculata* (L.) Roxb. leaves in alloxan induced diabetic rats. *Int J Pharm Tech Res*, **3(2):**747–56.
[47]. Prince PS, Kamalakkannan N, Menon VP (2004): Antidiabetic and antihyperlipidaemic effect of alcoholic Syzigium cumini seeds in alloxan induced diabetic albino rats. *J Ethnopharmacol* **91(2):**209–13.
[48]. Shajeela PS, Kalpanadevi V, Mohan VR (2012): Potential antidiabetic, hypolipidaemic and antioxidant effects of Nymphaea pubescens extract in alloxan induced diabetic rats. *J Appl Pharm Sci*, 83–8.
[49]. Sowmia C, Kokilavani R (2007): Antidiabetic and antihypercholesterolemic effect of Hemidesmus indicus Linn. R. root in Alloxan induced diabetic rats. *Ancient Sci Life*, **26 (4):**4.
[50]. Kliber J, Schindler I (1996): Recrystallization/precipitation behaviour in microalloyed steels. *J Mater Process Technol*, **60(1–4):**597–602.
[51]. Bailey CC (1949): Alloxan diabetes. *Vitam Horm*, **7:**365–82.
[52]. Mythili M, Vyas R, Akila G, Gunasekaran S (2004): Effect of streptozotocin on the ultrastructure of rat pancreatic islets. *Microsc Res Tech*, **63(5):**274–81.
[53]. Park BH, Rho HW, Park JW, Cho CG, Kim JS, Chung HT, et al. (1995): Protective mechanism of glucose against alloxan-induced pancreatic b-cell damage. *Biochem Biophys Res Commun* **210(1):**1–6.
[54]. Tasaka Y, Inoue Y, Matsumoto H, Hirata Y (1988): Changes in plasma glucagon, pancreatic polypeptide and insulin during development of alloxan diabetes mellitus in dog. *Endocrinol Japon*, **35(3):**399–404.
[55]. Elsner M, Gurgul-Convey E, Lenzen S (2008): Relation between triketone structure, generation of reactive oxygen species, and selective toxicity of the diabetogenic agent alloxan. *Antioxid Redox Signal*, **10(4):**691–700.
[56]. Bansal R, Ahmad N, Kidwai JR (1980): Alloxan-glucose interaction: effect on incorporation of 14 C-leucine into pancreatic islets of rat. *Acta Diabetol*, **17(2):** 135–43.
[57]. Jain D, Arya R (2011): Anomalies in alloxan-induced diabetic model: it is better to standardize it first. *Indian J Pharmacol*, **43(1):**91.
[58]. Elsner M, Hashimoto H, Nilsson T (2003): Cisternal maturation and vesicle transport: join the band wagon! *Molec Membr Biol*, **20 (3):**221–9.
[59]. Misra M, Aiman U, Alloxan (2012): An unpredictable drug for diabetes induction? *Indian J Pharmacol*, **44(4):**538.
[60]. Saravanan G, Ponmurugan P, Kumar GS, Rajarajan T (2010): Antidiabetic effect of S-allylcysteine: effect on plasma and tissue glycoproteins in experimental diabetes. *Phytomedicine*, **17 (14)):**1086–9.

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