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## Article

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## A New model for Alloxan-induced diabetes mellitus in rats

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### Abstract

**Background:** Alloxan is widely used to induce experimental diabetes mellitus (DM) in animals with different grades of disease severity by varying the dose of Alloxan used. This method has however be questioned by recent research work as an appropriate technique for the induction of diabetes. **Objective:** To provide a simple, yet concise and reproducible experimental procedure and model for Alloxan-induced DM in rats. **Methods:** The study was divided into 2 separate experiments. Experiment 1: Alloxan was administered, into four subgroups each (group 1- 100 mg of Alloxan /kg of rat body weight, group 2- 120 mg/kg, group 3- 150 mg/kg, and group 4- 170 mg/kg); in each subgroup, the dose of Alloxan was administered at different concentrations (20 mg/ml, 10 mg/ml, 5 mg/ml and 4 mg/ml) in groups of 10 rats each. The pre-induction fasting period was also varied between groups. Experiment 2: Following a pre-induction fasting period of 36 hours, animals received 150 mg Alloxan /kg body weight and at a concentration of 20 mg Alloxan/ml. **Result:** Alloxan administered intraperitoneally at 150 mg/kg of rat body weight, at 20 mg/ml and following a pre-induction fast period of 36 hours yielded the most favorably conditions with the least recorded mortality. **Conclusion:** From the results of this study, it can be concluded that alloxan is a diabetogenic drug with a strict protocol of use in inducing a predictable DM in rats and as such, this model is a standard and reproducible technique for the induction of DM in experimental rats.

**Keywords:** Alloxan, blood glucose, diabetes mellitus, pre-induction fast.

## Introduction

**D**M has been considered globally as one of the major health problem today<sup>1</sup>. The prevalence of DM has been shown to be progressively on the increase and prevalence of diabetes among adults is 6.4%, reaching 285 million adults in 2010 and possibly 439 million adults by 2030. Between 2010 and 2030, there will be an increase of 69% of diabetes prevalence in adults in developing countries, and a 20% increase in developed countries<sup>2</sup>. WHO projects that diabetes will be the 7th leading cause of death in 2030<sup>3</sup>. This suggests that studies must be carried out to provide adequate therapies and strategies to manage and curb the prevalence of the ongoing scourge. Research directed in this line is being carried out using up to date and sophisticated equipment in developed and some developing countries, with most developing countries barely trailing; all focused on providing adequate management therapies which will halt the progression of this disease and its associated complications.

Animal models of DM play a very important role to help us avoid unnecessary and ethically challenging studies in human subjects, as well as to obtain a comprehensive scientific viewpoint of this disease. Also, due to various models of possible therapeutic interventions, and associated complications that can arise, it is best to simulate DM in experimental animals; these provide alternative safer models to which the therapeutic intervention can be administered.

Although there are several methods of inducing DM, chemical methods of Alloxan and streptozotocin induced DM represent the most important and highly preferable experimental models for this pathological condition<sup>4</sup>.

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is the most common chemical compound used to induce experimental diabetes due to its selective destruction of beta cells in the pancreatic islets through sequential changes leading ultimately to apoptosis<sup>4</sup>. The dose of alloxan used to induce diabetes varies according to different species of animals such as rat: 40-200 mg/kg intravenously (i.v.) or intraperitoneally

(i.p.)<sup>5,6</sup>. Alloxan has two distinct pathological effects: it selectively inhibits insulin secretion induced by glucose, through its ability to generate reactive oxygen species (ROS) resulting in the selective necrosis of beta cells<sup>7</sup>.

Alloxan has been widely used to induce experimental DM in animals such as rabbits, mice and dogs with different grades of disease severity by varying the dose of Alloxan used<sup>8</sup> causing "Alloxan Diabetes", a form of insulin-dependent diabetes mellitus similar to type 1 diabetes<sup>9,10</sup>.

Alloxan induces a triphasic blood glucose response when injected into experimental animals<sup>11</sup>. It is affordable and readily available, and as such, a most popular compound for inducing DM in most developing countries. However, recently there is a lot of controversy has been created over its use in the induction of DM. Recent studies have questioned the technique of induction, the duration of induced hyperglycemia and even the use of Alloxan as a diabetogenic agent. This study therefore seeks to address this particular challenge faced by scientists and researchers, with the aim to providing a simple, yet concise and reproducible experimental procedure and model for Alloxan-induced DM in rats.

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## Methods

### Animals:

This study was carried out in the Department of Physiology, University of Ilorin, Nigeria, from October 2017 to February 2018. Male Sprague Dawley rats (10-12 weeks and weighing about 200-220g) were obtained from the animal house of the College of Health Sciences, University of Ilorin. They were kept in plastic cages and allowed free access to feed and water throughout (except during the period of pre-induction fast) the experimental period. Animal identification was done in the Department of Cell Biology and Genetics, University of Lagos. All guidelines with the use and care of laboratory animals were strictly adhered to in accordance with the Institutional Animal Care Use Committee (IACUC, 2002)

**Induction of Diabetes Mellitus:**

DM was induced in the morning after fasting (Pre-induction fast PIF) the animals for 12 hours and 36 hours (depending on the animal group) by a single intraperitoneal injection of Alloxan monohydrate (Sigma Aldrich, Inc, USA) dissolved in normal saline. The blood glucose levels of the rats were checked 48 hours and 72 hours later, and those with blood glucose levels above 11mmol/L were considered diabetic.

**Determination of blood glucose:**

To assess the effect of Alloxan and to chemically establish the diabetic state, blood sample was obtained from the tip of the rat tail at forty-eight (48) and seventy-two (72) hours after injection of Alloxan. A sample of the rat's blood was collected on a reagent strip to determine the blood glucose level using a portable glucometer (Arcu Check Inc. Carlifornia, USA).

**Experimental procedure:**

The study was divided into two (2) separate experiments; experiment I was used to determine the most appropriate conditions (dose of Alloxan/kg of rat body weight, concentration of Alloxan dissolved in normal saline, and pre-induction fast hours) for inducing diabetes with alloxan, while the subsequent experiment served to confirm the results from experiment I.

**Experiment I:**

Animal grouping

Three hundred and forty rats (340), weighing between 200-220 g, were used in this Experiment,

which consisted of two major groups, based on the hours for which the experimental rats were fasted before induction (group A- 12 hours pre-induction fast and group B-36hours pre-induction fast), with each group further subdivided, based on dose of Alloxan administered, into five subgroups each (group 1- Normal Saline (Control), group 2- 100 mg of Alloxan /kg of rat body weight, group 3- 120 mg/kg, group 4- 150 mg/kg, and group 5- 170 mg/kg). In each subgroup, dose of Alloxan was then administered at different concentrations (20 mg/ml, 10 mg/ml, 5 mg/ml and 4 mg/ml) in groups of 10 rats each. This is represented in Table 1

**Experiment II:**

Experimental rats were divided into two groups of ten rats each. Group I served as the control group (normal saline) while group II served as the experimental group (of normal saline) 150 mg Alloxan /kg body weight and at a concentration of 20 mg Alloxan/ml

**Statistical Analysis:**

Values are reported as mean±standard error of mean (SEM) and were analysed by One-way ANOVA, followed by Students' Newman-Keuls Post-hoc test in Experiment I and Students' t-test in Experiment II, using the GraphPad Prism, version 6 Software (2013). Differences were considered statistically significant at  $p \leq 0.05$ .

**Table I:** Experiment I animal grouping according to dose of Alloxan administered intraperitoneal and duration of pre- induction fast (N=340)

Experiment I Grouping									
Group A- (n=170) 12 Hours Pre-induction Fast					Group B- (n=170) 36 Hours Pre-induction Fast				
Control (n=10)	100mg/kg (n=10)	120mg/kg (n=10)	150mg/kg (n=10)	170mg/kg (n=10)	Control (n=10)	100mg/kg (n=10)	120mg/kg (n=10)	150mg/kg (n=10)	170mg/kg (n=10)
	20mg/ml	20mg/ml	20mg/ml	20mg/ml		20mg/ml	20mg/ml	20mg/ml	20mg/ml
	10 mg/ml	10 mg/ml	10 mg/ml	10 mg/ml		10 mg/ml	10 mg/ml	10 mg/ml	10 mg/ml
	5 mg/ml	5 mg/ml	5 mg/ml	5 mg/ml		5 mg/ml	5 mg/ml	5 mg/ml	5 mg/ml
	4 mg/ml	4 mg/ml	4 mg/ml	4 mg/ml		4 mg/ml	4 mg/ml	4 mg/ml	4 mg/ml

## Results

### Experiment I

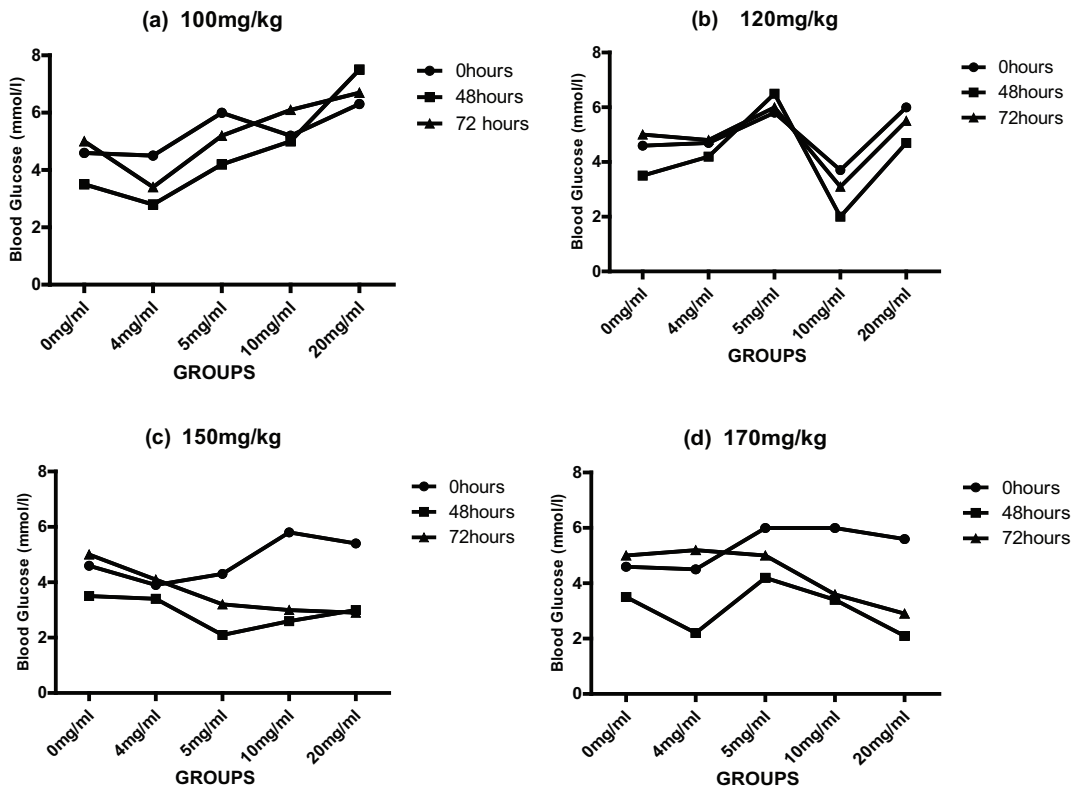
Blood glucose levels in Alloxan-induced rats following 12 hours PIF

*Alloxan Dosage-100mg/kg:* The highest blood glucose level recorded was  $7.5 \pm 0.095$  mmol/L. This was recorded following administration at a concentration of 20 mg/ml after 48hrs post-induction. The least value ( $2.8 \pm 0.034$  mmol/L) was also recorded 48 hrs post-induction but at a concentration of 4 mg/ml. The remaining recorded blood glucose levels fell between this range (figure 1a).

*Alloxan Dosage-120 mg/kg:* Values taken between 0 hrs-72hrs post-induction fell between

the range of  $2.0 \pm 0.042$  mmol/L and  $6.50 \pm 0.062$  mmol/L across all groups (figure 1b). Only one rat died during the experimental period and this was recorded in the 20 mg/ml group (figure 3)

*Alloxan Dosage-150 mg/kg:* Following the administration of 150 mg/kg at different concentrations (4 mg/ml, 5 mg/ml, 10 mg/ml and 20 mg/ml) (figure 1c), the recorded blood levels fell well beneath the considered value for DM (11.0 mmol/L), with the highest ( $4.1 \pm 0.052$  mmol/L) observed in rats administered at a concentration of 4 mg/ml and the least ( $2.1 \pm 0.060$  mmol/L) in rats at 5 mg/ml. Only one (1) rat died in this group during the experimental period (figure 3).



**Figure 1:** Blood glucose levels at 0 hour, 48 hours and 72 hours in experimental rats following intraperitoneal administration of Alloxan (a) 100 mg/kg of rat tissue, (b) 120 mg/kg of rat tissue, (c) 150 mg/kg of rat tissue and (d) 170 mg/kg of rat tissue; after 12 hour PIF, at different concentrations

*Alloxan Dosage-170 mg/kg:* Figure 1(d) shows the recorded blood glucose levels in rats following administration of Alloxan at a dosage of 170 mg/kg at 0hrs, 4hrs and 72hrs post-induction. During the experimental period, 2 rats died following administration at a concentration of 10 mg/ml and 3 at 20 mg/ml (figure 3).

Blood glucose level in Alloxan-induced rats with 36 hours (PIF)

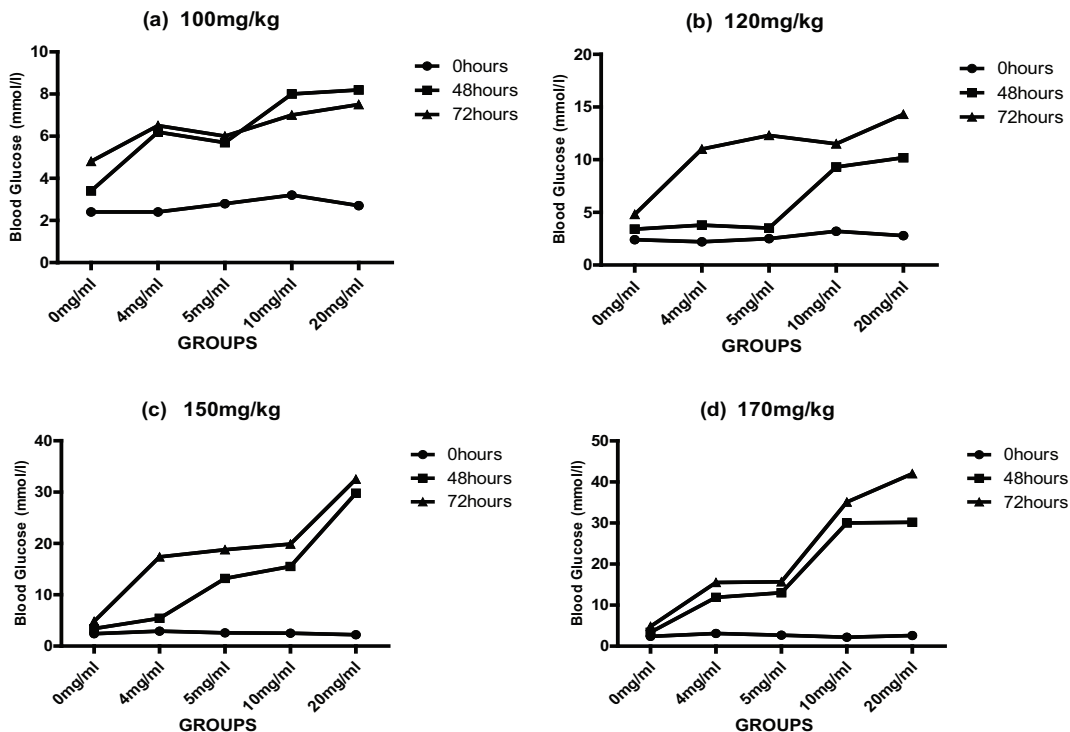
*Alloxan Dosage-100mg/kg:* The highest blood glucose ( $8.2 \pm 0.920$  mmol/L) was recorded 48hrs post induction at a concentration of 20 mg/ml (figure 2a). There was no recorded animal death throughout the experimental period (figure 3).

*Alloxan Dosage-120mg/kg:* Blood glucose levels considered were recorded following intraperitoneal injection of Alloxan, 72hrs post-induction at 4 mg/ml ( $11.0 \pm 0.194$  mmol/L), 5 mg/ml ( $12.3 \pm 0.205$  mmol/L), 10 mg/ml ( $11.5 \pm 0.225$  mmol/L) and 20 mg/ml ( $14.3 \pm 0.256$  mmol/L)(fig

2b). Five (5) deaths were recorded with two (2) in the 20 mg/ml concentration group and one (1) each in the remaining groups (figure 3).

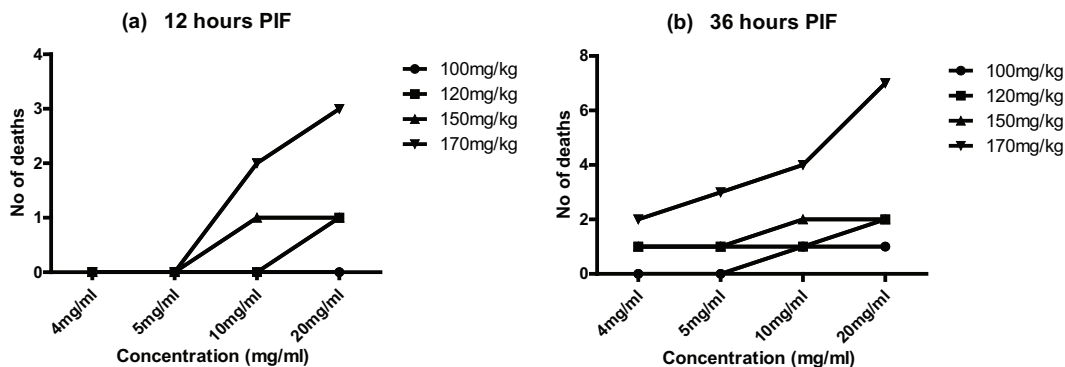
*Alloxan Dosage-150mg/kg:* Blood glucose levels recorded 48hrs and 72 hrs post-induction in all groups were diabetic with the highest value ( $32.5 \pm 0.25$ mmol/L) recorded at a concentration of 20 mg/ml. The least diabetic value was ( $13.2 \pm 0.202$ mmol/L) recorded 48hrs post-induction at a concentration of 5 mg/ml(figure 2c). Only two (2) of the experimental rats died following administration at 10 mg/ml(figure 3).

*Alloxan Dosage-170mg/kg:* At 48hrs post-induction, all experimental rats were diabetic with blood glucose levels ranging between  $11.9 \pm 0.262$  mmol/L and  $42.0 \pm 1.092$  mmol/L. Recorded death in this group was sixteen (16) with seven (7) in the 20 mg/ml, four (4) in the 10 mg/ml, three (3) in the 5 mg/ml and two (2) in the 4 mg/ml concentration groups.



**Figure 2:** Blood glucose levels at 0 hour, 48 hours and 72 hours in experimental rats following intraperitoneal administration of Alloxan (a) 100 mg/kg of rat tissue, (b) 120 mg/kg of rat tissue, (c) 150 mg/kg of rat tissue and (d) 170 mg/kg of rat tissue; after 36 hours PIF, at different concentrations.

No of deaths

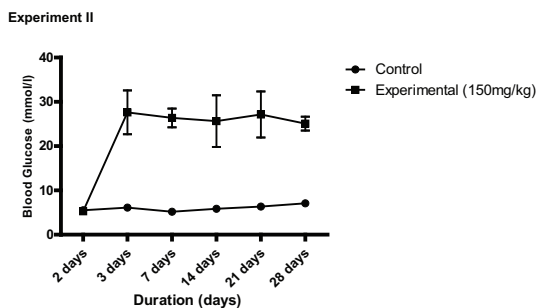


**Figure 3:** Number of deaths in experimental rats following intraperitoneal administration of Alloxan after (a) 12 hours PIF and (b) 36 hours PIF, at different concentrations.

### Experiment II

The results from experiment II is shown in fig 4. Blood glucose levels were recorded in rats after intraperitoneal administration of Alloxan at a dosage of 150 mg/kg and at a concentration of 20 mg/ml; pre-induction fast was 36hrs. The highest blood glucose level ( $27.6 \pm 0.430$  mmol/L) on day 3 post-induction, followed by day 21 ( $27.2 \pm 0.524$  mmol/L) and then on day 7 ( $26.4 \pm 0.650$  mmol/L). The least value ( $5.3 \pm 0.095$  mmol/L) was recorded on day 2

### Experiment II



**Figure 4:** Blood glucose level (mmol/L) in control and experimental rats (Experiment II) following intraperitoneal administration of Alloxan (150 mg/kg of rat tissue) following a 36 hour PIF, and at a concentration of 20 mg/ml.

### Discussion

This study was carried out to obtain a standard and reproducible model for Alloxan-induced DM in rats. Alloxan has two distinct pathological effects; it selectively inhibits insulin secretion induced by glucose, and causes a state of insulin dependence through its ability to generate reactive oxygen species, resulting in the selective necrosis of beta cells<sup>7</sup>.

From this study, a dose of 150 mg of Alloxan per kg of rat tissue, at a concentration of 20 mg of Alloxan per ml of normal saline, administered intraperitoneally, following a 36 hour period of PIF, yielded the most adequate conditions for the induction of DM in rats; and though the success rate of induction was less than in the groups with a higher dosing of Alloxan, which had a higher mortality rate. These findings are similar to the results of others<sup>12</sup>, but in their research, animals were deprived of food for 48 hours and administration of Alloxan was given intravenously.

Yonardy and Colak<sup>13</sup> also used a similar dosage of Alloxan monohydrate (150 mg/kg) and route of administration (intraperitoneally), following an 18 hour PIF to induce hyperglycemia in experimental rats, but the duration of the induced hyperglycemia was not recorded or taken into consideration.

Findings by Monika and Umme<sup>14</sup> strongly contradict the results of this study. They suggested that Alloxan is an unpredictable drug for the induction of DM due to inconsistencies associated with the use of Alloxan in induction and maintenance of a stable diabetic state in both rats and rabbits. They went ahead to support the viewpoint of Dinesh and Kumar<sup>15</sup> who also stated that there were inconsistencies in doses of drugs, routes of administration, duration and severity of diabetes and methodology in alloxan-induced diabetic models, which made its accuracy controversial. A pitfall to their research though was the period of PIF in the experimental animals, which was just an overnight fast (corresponding to 12 hours).

### Conclusions

We conclude from our findings that Alloxan monohydrate is indeed a diabetogenic drug that has a strict protocol of use in inducing a predictable DM in rats. Current technique for inducing this condition in animal models should be continuously upgraded upon to provide more accuracy to achieve a better outcome of this experimentation on diabetes. We hereby propose our model as a standard and reproducible technique for the induction of DM in experimental rats.

**Conflict of Interest** The authors have no conflict of interest among them

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