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INDUCTION OF EXPERIMENTAL DIABETES IN WISTAR RAT BY ALLOXAN: A STUDY FOR THE STANDARDIZATION OF EFFECTIVE DOSE

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ABSTRACT

The present investigation was undertaken to evaluate the effective dose for the induction of diabetes by alloxan in Wistar rats. Alloxan and streptozotocin (STZ) are the most widely used in diabetes studies, and alloxan is the most affordable. Alloxan is a glucose analogue-like structure; it enters the β cell of the endocrine pancreas through the GLUT2 glucose transporter and destroys it by forming ROS (reactive oxygen species), and insulin hormone secretion is hampered. Alloxan causes a condition known as "Alloxan Diabetes," like human type 1 diabetes. But in some cases, due to the short half-life of alloxan, which will spontaneously decompose into alloxanic acid in an aqueous solution within minutes, alloxan is therefore non-diabetic and non-responsive. Few-induced animals are auto-reversed from diabetic to normal animals within a few days after the induction of alloxan due to the regeneration of β cells. In some cases, alloxan is non-responsive and cannot induce diabetes because young animals are highly resistant to the toxic effects of alloxan. So, we needed to find out the best and most successful dose selection for the initiation of diabetes in the Wister rat under laboratory conditions. In our study, intraperitoneal administration of 150 mg of alloxan per kilogram of rat body weight produced the most adequate conditions for inducing diabetes mellitus in rats. The induction success rate was lower in the higher group (160–170 mg/kg b.w.) and had a higher mortality rate. Lower doses of alloxan (110–140 mg/kg b.w.) can result in fewer diabetic

animals, a high number of non-responsive animals, and a high rate of auto-reversion for diabetics to a normoglycemic state within seven days.

Keywords: Diabetes, Alloxan, Streptozotocin, ROS, β cell, intraperitoneal

INTRODUCTION:

The pancreas is made up of 2% endocrine and 98% exocrine tissue. The islets of Langerhans are responsible for the formation of pancreatic endocrine cells. The islets of Langerhans are made up of four different types of cells: α, β, δ and PP (pancreatic polypeptide) cells. These cells secrete glucagon, insulin, and somatostatin hormones, as well as PP cells, which secrete pancreatic polypeptides **[1]**. By stimulating the β cell to release insulin, glucose stimulates the β cell and promotes glucose uptake and storage throughout the body. Diabetes mellitus (DM) demonstrates itself as abnormal carbohydrate, protein, and fat metabolism due to either whole or proportional inadequacy of insulin hormone discharge or insulin hormone activity **[2]**. To get out of futile and justly significant findings on humans, as well as to find a thorough and efficient assessment on this disease, animal models of diabetes mellitus are very valuable. In addition, due to the wide range of possible pharmaceutical intrusions and the potential barriers associated with them, it is best to stimulate diabetes mellitus in investigational animals. These models deliver a better framework within which healing interventions can be used. Alloxan and streptozotocin (STZ) are the most often used diabetogenic drugs in diabetes research. Other recognized diabetogenic agents include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load, and anti-insulin serum.

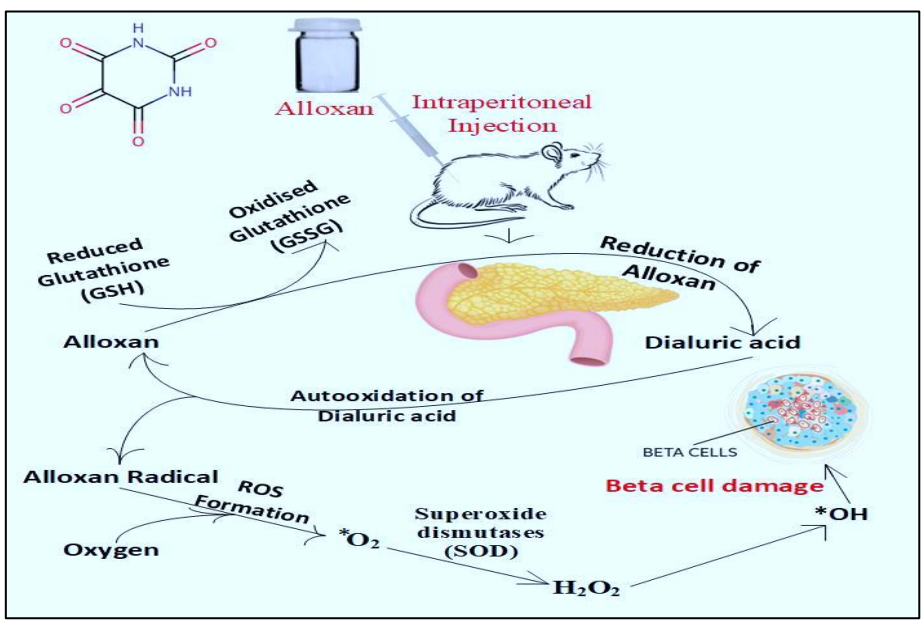

Figure 1: Mechanism of alloxan-induced diabetes based on Ighodaro *et al.*, 2017 [12]. Alloxan is reduced to dialuric acid and re-oxidized to alloxan producing alloxan radicals and reactive oxygen species (ROS) which undergo dismutation (by superoxide dismutase, SOD) to form hydrogen peroxide (H_2O_2). Side reactions can also result in the formation of hydroxyl radicals (*OH). These *OH trigger DNA fragmentation in -cells, which results in apoptosis

Alloxan is more likely to be utilized than STZ because of its lower price and greater accessibility **[3]**. The molecular formulae of alloxan are $C_4H_2N_2O_4$ and it has a 142.06 Da molecular mass. Brugnatelli used alloxan for the first time in 1818, and Wohler *et al.* **[4, 5]** described it. Its use in the induction of diabetes in rabbits was first described by Dunn *et al.* in 1943 **[6]**. Alloxan is a pyrimidine derivative that is structurally similar to glucose and has become of interest in diabetes research as it has been classified as a diabetogenic agent. Alloxan, on the other hand, cannot cross the plasma membrane because of its hydrophilic nature. However, alloxan is able to enter cells due to its glucose-like structure. Alloxan is a poisonous glucose analogue that destroys β cells in the pancreas. The GLUT2 glucose transporter is used to transfer alloxan into pancreatic beta cells. When thiols are present intracellularly with alloxan, ROS (reactive oxygen species) are generated in a recurrent reaction with the reduction of dialuric acid that occurs. In this redox process, free radicals are the ones that make alloxan toxic to beta cells **(Figure 1) [7]**. The central 5-carbonyl group of alloxan can react with the thiol groups of various enzymes in cells, particularly glucokinase, which is the most well-known and sensitive of the thiol enzymes. This is yet another significant role that alloxan plays in the development of diabetes. After a period of seventy-two hours, animals whose blood glucose levels are more than ≥ 250 mg/dl will be classified as diabetic and may participate in the diabetes-related research. But in some cases, due to the short half-life of alloxan, which will spontaneously decompose into alloxanic acid in an aqueous solution within minutes, alloxan is therefore not diabetogenic and non-responsive **[7, 8, 9]**. Sometimes auto reversal from diabetic to normal animals occurs within a few days after the induction of alloxan. Alloxan has been extensively applied to induce investigational diabetes in small mammals such as mice, rats, rabbits, and dogs with varying levels of seriousness using varying doses of Alloxan, causing a condition known as "Alloxan Diabetes," like human type 1 diabetes. The amount of alloxan utilized to bring on diabetes varies from animal to animal, such as the rat: 40–200 mg/kg injected intraperitoneally or intravenously **[10, 11]**. The aims of our study were to find out the best and most successful dose selection for the initiation of diabetes in the Wister rat under laboratory conditions.

MATERIALS AND METHODS:

After receiving institutional consent, experimental research was undertaken at the Endocrinology laboratory of the Zoology Department at the University of Kalyani in West Bengal, India. Adopting the parameters given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

guidelines **[12]** for the treatments and study to be conducted on albino rats. This research used Wistar rats weighing between 120 and 150 grams that were in good health. All albino rats were housed in cages that were clean, dry, and had a 12-hour light-dark phase at room temperature and relative humidity. They were acclimated to the accessible living circumstances, fed a normal laboratory meal, and provided with water on demand. The floors of the cages were covered with a covering of dry grass to absorb rat urine.

Induction of Diabetes Mellitus:

Induction of alloxan diabetes was done thus in the morning, subsequently fasting the animals for 12 hours by IP (intraperitoneal) injection of Alloxan monohydrate (Sigma Aldrich, Inc., USA). The rats will be injected with alloxan monohydrate in saline (0.9% NaCl) at a single dose of 110 mg/kg b.w., 120 mg/kg b.w., 130 mg/kg b.w., 140 mg/kg b.w., 150 mg/kg b.w., 160 mg/kg b.w., and 170 mg/kg b.w. intraperitoneally (IP) at the lower right quadrant of the abdomen towards the head at 45° angle **(Figure 2)**. After one hour of alloxan administration, the animals will be fed 5% dextrose solution ad libitum for 24 hours to overcome the early hypoglycemic shock. After the induction of alloxan for seven days, all animals' fasting blood glucose levels, behavioural changes, body weight, and mortality were monitored. Blood samples were collected from the tail vein.

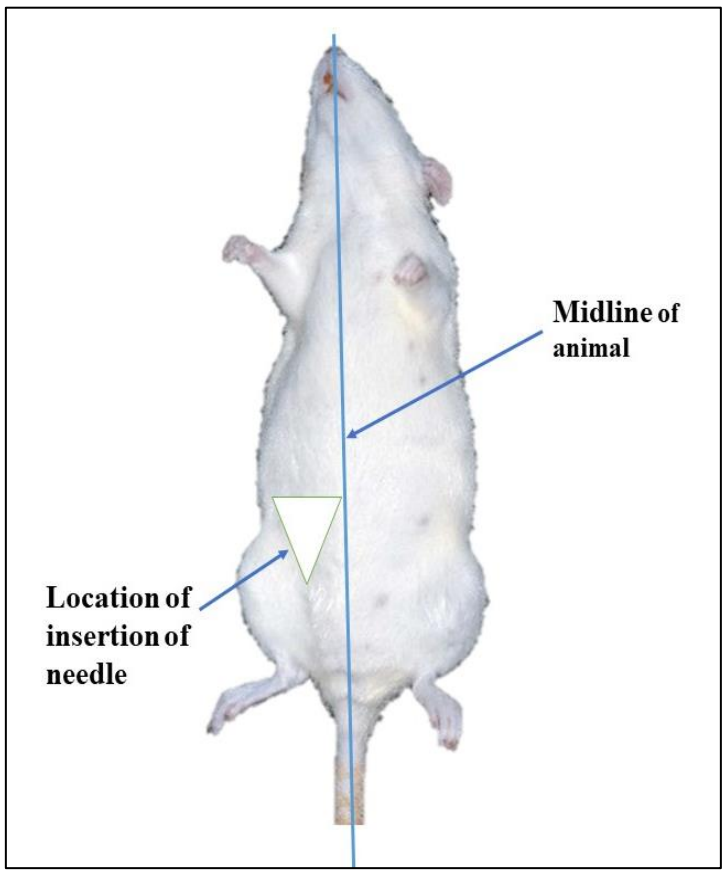

Figure 2: Site and location of intraperitoneal injection in rats for induction of diabetes by alloxan. The injection will be done at the lower right quadrant of the abdomen toward the head at a 30-45° angle

Experimental Design:

The animals are divided into eight groups, with 10 animals in each group. In this experiment, we used Wistar rats to find out how much alloxan to give per kg of body weight to cause diabetes that would be permanent.

Groups Index	Dose of Alloxan	Observation (period -7days)
TC	Nil	Mortality Fasting blood glucose levels Nonresponsive to alloxan Auto reversal from diabetic to normal animal.
TA1	110 mg/kg b.w.	
TA2	120 mg/kg b.w.	
TA3	130 mg/kg b.w.	
TA4	140 mg/kg b.w.	
TA5	150 mg/kg b.w	
TA6	160 mg/kg b.w	
TA7	170 mg/kg b.w	

RESULT AND DISCUSSION:

To evaluate the effects of Alloxan and chemically identify the diabetes condition, blood samples were obtained from the vein at the tip of the rat's tail on a daily basis from the 2nd day up to 7 days after the injection of Alloxan. Day seven fasting glucose levels ≥ 250 mg/dl are considered diabetic, which are allowed to participate in the diabetic-related experimental study. Fasting glucose levels ≤ 249 mg/dl are considered non-diabetic. The animals with higher glucose at early days ≥ 250 mg/dl and later it was auto-reversed into ≤ 249 mg/dl are considered an auto-reversal group. Animals whose blood glucose levels were in the normal range before and after induction with alloxan are in the non-responsive group. The fasting glucose content was measured using a compact glucometer after a blood sample from the rat's tail vein was taken on a reagent strip (Accu Check Inc., California, USA). A random cross-check of blood glucose levels was done through the glucose-oxidase peroxidase (GOD POD) method (Trinder method) **[14]**. A number of studies have questioned alloxan's effectiveness, safety, and toxicity in the production of experimental diabetes. Ighodaro *et al.* have shown that the alloxan-induced diabetes model has some oddities and inconsistencies **[12]**. This has made us worry and pay more attention to how it is used to cause diabetes. The auto-reversal and instability of alloxan-induced hyperglycemia are of particular concern. Alloxan has two different clinical effects: it selectively inhibits glucose-induced insulin discharge and causes an insulin-dependent state through its ability to produce ROS (reactive oxygen species), leading to selective necrosis of β cells **[7]**. In this study, after 12 hours of fasting, intraperitoneal administration of 150 mg of alloxan per kilogram of rat body weight produced the most adequate conditions for inducing diabetes mellitus in rats; although the induction success rate was lower than the alloxan dose in the higher group, the latter had a higher mortality rate. Lower doses of alloxan (110–140 mg/kg b.w.) can result in

mild diabetes (blood glucose levels between 120 mg/dl and 249 mg/dl) or reversion to a normoglycemic state **(Table 1 and Figure 3)**. In several investigations, the dosage of alloxan used to induce diabetes was proven to be inappropriate. According to these investigations, alloxan induces a very mild form of diabetes. However, after a week, the blood glucose levels of these animals returned to normal. Cells of the Islet of Langerhans in rats have the ability to regenerate **[15]**. Thus, the illusion is created that the experimental substance has an anti-diabetic effect. The half-life of alloxan is less than one minute, so it may be rapidly destroyed in the body and cannot destroy beta cells in the pancreas **[16]**. Some rats are highly resistant to the toxic effect of alloxan **[17]**. Induction of alloxan can cause damage to the kidney's renal tubules, which causes an alteration of the blood glucose level. Auto-recovery from diabetes to normal blood sugar levels is thought to be the result of a proliferation of beta cells in either the exocrine section of the pancreas or the intestinal duct epithelium **[18]**. Because of this, rats that have been given alloxan and become diabetic recover from their diabetic condition and become normoglycemic. At a dose of 150 mg/kg b.w. or higher, our experimental investigation showed that non-responsiveness and auto-reversion could be completely avoided. The optimal dosage of alloxan for rats is 150 milligrams per kilogram of body weight. After 18 hours of fasting, Yanarda and çolak **[19]** administered intraperitoneal doses of alloxan monohydrate (150 mg/kg) to produce hyperglycemia in experimental rats, but they did not monitor or account for the duration of this condition.

Table 1: Effects of alloxan in the Wistar rat: The rates of mortality, diabetes, non-responsiveness, and auto-reversal in the animals are represented as percentages (%) of various doses of alloxan induction as well as the control group

Animal Group	TC	TA1	TA2	TA3	TA4	TA5	TA6	TA7
Mortality %	0	0	20	20	30	40	60	80
Diabetic %	0	10	40	40	50	60	40	20
Nonresponsive %	0	70	20	10	10	0	0	0
Auto reversal %	0	20	20	30	10	0	0	0

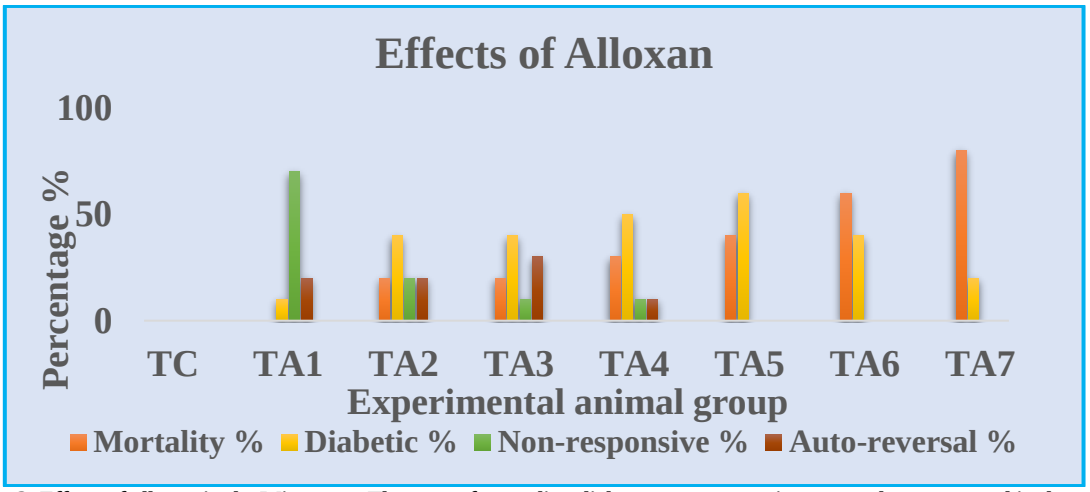

Figure 3: Effects of alloxan in the Wistar rat: The rates of mortality, diabetes, non-responsiveness, and auto-reversal in the animals are represented as percentages (%) of various doses of alloxan induction as well as the control group

CONCLUSION

There are differences in the doses of drugs, how they are given, how long and how severe the diabetes is, and how they were made, which makes it challenging to say how accurate they are. In the body, alloxan is broken down very quickly. Its half-life is less than one minute. Intraperitoneal routes of administration would delay the onset of alloxan action. Young animals may have a high level of resistance to toxic alloxan. The auto-recovery from diabetes to normoglycemia is due to the proliferation of β cells or rejuvenation of the intestinal duct epithelium or the exocrine section of the pancreas. Inducing diabetes with a quick intravenous injection has been shown in several studies to have a high percentage of effectiveness; however, this method is also linked to a very high fatality risk. Before starting an experiment on the pharmacokinetic and pharmacodynamic profile of alloxan, we should standardize it first.

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