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RESEARCH ARTICLE

A histological study of alloxan-induced diabetes on experimental male Wistar rats

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ABSTRACT

Background: Alloxan is a toxic chemical which is used for induction of diabetes in experimental animals. In the light of various earlier researches which showed different dosages for induction, it has been decided to select and optimize the correct dosage. Alloxan which is a urea derivative is known for its diabetogenic action and causes damage to β-cells. A histological study is conducted for dosage selection of alloxan causing diabetes.**Aims and Objectives:** This study strives to establish the correct dose which is 120 mg/kg body weight (b.w) intraperitoneally to Wistar rats. **Material and Methods:** Male albino rats were procured and grouped as Group 1 (control), and alloxanized groups as Group II: 90 mg/kg b.w, Group III: 120 mg/kg b.w, and Group IV: 150 mg/kg b.w the biochemical parameters like blood glucose, urea, creatinine, uric acid, etc., were determined. Animals were sacrificed and 5 mm pancreatic tissues from various groups were processed for histological examination. **Results:** Among all, a significant difference in biochemical parameters was observed with 120 mg/kg b.w (Group III) when compared to control group. There was marked degeneration and necrosis in the pancreatic β-cells of islets. **Conclusion:** Although earlier works have studied the diabetic effect of alloxan, it is pertinent to optimize and standardize the dose selection of alloxan with desirable effects.

KEY WORDS: Alloxan; β-cells; Diabetes; Intraperitoneal; Islets of Langerhans; Pancreas

INTRODUCTION

Diabetes mellitus is the leading cause of morbidity with its attendant complications and is on the rise not only in the developed countries but also in developing countries. The necessity of developing an effective treatment to control this metabolic disorder is of paramount importance. To achieve the objective, studies on experimental albino rats were performed by inducing diabetes with hyperglycemic drug: Alloxan in different doses to achieve a stable diabetic state.

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Alloxan has a selective action on the β-cells of the islets of Langerhans and is widely used for studies by many workers.[1,2] The intraperitoneal route of alloxan is the most widely used method of induction of diabetes in Wistar rats because of its structural similarity to glucose, the structural integrity of the cytoskeleton, lysosomes, DNA and mitochondria would be lost, and the β-cells disintegrate resulting in a lack of insulin production when alloxan is taken up by the β-cells.[3-7]

The dosage of alloxan necessary to induce desirable level of diabetes had to be determined by optimization in view of high incidence of mortality of experimental animals following high dosage (>50%).[8] Alloxan causes a massive reduction in insulin release by the destruction of the β-cells

of the islets of Langerhans, inducing hyperglycemia.[9] Insulin deficiency leads to various metabolic alterations in the animals, namely, increased blood glucose, increased cholesterol, increased levels of alkaline phosphate, and transaminases.[10,11] The resultant metabolic derangement affects the liver as well as leading to an alteration of the enzymes elaborated from the parenchyma. It leads to increased bilirubin in the serum and a decrease in the total protein, especially the albumin fraction. Alloxan induces the formation of reactive oxygen species (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) which mediate cellular damage.[12] This cellular damage may in addition induce autoimmune reactions against the β-cells. Second, alloxan disrupts the formation of microtubules as well as destroying those already formed.[13] Third, alloxan is thought to inhibit the enzyme O-linked n-acetyl glucosamine transferase,[14] which is very abundant in the β-cells and catalyzes protein O-glycosylation. For these reasons, alloxan is a potent diabetogenic agent and is used widely to induce diabetes in experimental animals.[15]

Three different doses of alloxan were used in the present pilot study to standardize the dosage of alloxan for the induction of diabetes. There is no consensus regarding the amount of alloxan which is required to achieve adequate hyperglycemia. The margin of safety of alloxan was not established in earlier experimental works. This study strives to establish the correct dose which is 120 mg/kg body weight (b.w) intraperitoneally to Wistar rats.

MATERIALS AND METHOD

The experiments were performed with the prior approval of the institutional animal ethics committee and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. CPCSEA No. 753/03/C/CPCSEA.

Wistar albino male rats weighing 280-290 g are utilized for the present study. Experiments were performed with the permission of the institutional ethics committee.

In the present study, male Wistar rats were used and grouped as follows to conduct a model development of diabetes:

- Group I: Control rats received 1 ml of normal saline only ($n = 3$).
- Group II: Rats received 90 mg/kg b.w in a single dose of alloxan intraperitoneally ($n = 3$).
- Group III: Rats received 120 mg/kg b.w in a single dose of alloxan intraperitoneally ($n = 3$).
- Group IV: Rats received 150 mg/kg b.w in a single dose of alloxan intraperitoneally ($n = 3$).

All rats were kept under observation for 1 week before the experiments to permit the animals to adjust to the environment. All animals were fed standard rat pellets and were provided tap water to drink ad libitum. They were housed in a facility with 12-12 h light-dark cycle that is maintained at 25°C. All animals were weighed before the injections. At the end, animals were anesthetized with ether inhalation and sacrificed.

Blood Collection

Under aseptic conditions, blood samples were collected on the first day before inducing alloxan and later on the 3rd, 7th, and 14th days of alloxanization from the tail vein. Blood glucose determination was performed by one touch glucometer strip test. Blood was collected from the retro-orbital plexus for biochemical assay before all animals were sacrificed after 28 days. Blood samples were centrifuged for 10 min at 3000 rpm within an hour of sample collection and the serum was obtained. All the specimens of sera were stored at −40°C until use.

Biochemical Parameters

Determinations were done for the following parameters: Blood glucose, urea, creatinine, uric acid, albumin, total protein, bilirubin, lipid profile, and liver enzymes:

Histological Examination

Isolation of the pancreas and tissue processing

The abdominal cavity is exposed following a median incision. All abdominal viscera were identified, dissected, and preserved in 10% formalin for further use. The pancreas was quickly isolated by fine dissection and immediately preserved in 10% formalin. 5 mm pancreatic tissue samples from all groups were fixed in 10% neutral buffered formalin and processed for histological examination.

The sections were stained with hematoxylin and eosin and examined under a light microscope at 100 and 400 magnifications.

RESULTS

Loss of b.w is a characteristic feature of diabetes mellitus due to improper utilization of glucose. No significant weight loss was observed in Group II Wistar rats treated with 90 mg/kg b.w of alloxan. A steady decrease in b.w in Group III treated with 120 mg/kg b.w of Alloxan was seen. A drastic loss of b.w in Group IV was evident, which was treated with 150 mg/kg b.w of alloxan over a period of 28 days (Table 1).

It was observed that in comparison to control group, alloxan-induced diabetes groups have shown significant differences

in the biochemical values of the analytes. There was sustained hyperglycemia throughout the period of study. Lipid profile and liver enzymes showed a marked increase. Serum urea, creatinine, and uric acid were significantly elevated compared to those of control group of rats (Table 2).

The serum glucose, bilirubin, and albumin levels in Group II, Group III, and Group IV have shown a consistent elevation when compared to the control group and differed significantly from one another.

The lipid profile of Group II, Group III, and Group IV has shown a consistent increase in cholesterol, low-density lipoprotein (LDL), very LDL (VLDL), and TGs and a decrease in high-density lipoprotein (HDL) levels in IaIloxan-treated rats in comparison to control group (Tables 2 and 3).

The liver enzymes of Group II, Group III, and Group IV showed a consistent increase of aspartate aminotransferase/serum glutamic oxaloacetic transaminase (SGOT), alanine transaminase/serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase levels in alloxan-treated rats in comparison with control group (Table 3).

In the kidney function tests, no appreciable increase in the levels of creatinine, urea, and uric acid with a dose of 90 mg/kg b.w occurred. Whereas at 120 mg/kg b.w dosage, a significant increase in urea, creatinine, and uric acid levels was observed compared to control group which reflected the renal impairment due to hyperglycemia. A dose of 150 mg/kg b.w resulted in azotemia leading to death of the rat within a week.

Histological observations

Group I

H and E stained tissue at 400 magnification showed normal pancreatic architecture which was uniform throughout. The islets were full of centrally placed β-cells which stained pale blue with H and E. In the periphery alpha cells which are relatively smaller in size, with deeply stained nuclei,

Table 1: Comparison of b.w between four groups

Groups	b.w (g)				
	0 day	3rd day	1st week	2nd week	4th week
Group I	286.67±5.77	286.67±5.77	286.67±5.77	291.67±5.77	295.33±6.42
Group II	285±5	285±5	283±5	280.67±5.13	277.67±6.65
Group III	286.67±5.77	286.67±5.77	279.67±5.85	258.67±6.65	228.33±6.42
Group IV	290	286.67±5.77	272.33±6.42	252.33±7.63	154.67±109.73

Mean, standard deviation and one-way ANOVA was done to know the significance. Values were expressed as means±S.D Using one-way ANOVA, the results were significant at 0.00., b.w: Body weight

Table 2: Showing biochemical parameters of different doses of alloxan when compared to control

Parameter	Mean±SD			
	Control Group I rats	Alloxan 90 mg/kg. b.w Group II rats	Alloxan 120 mg/kg. b.w Group III rats	Alloxan 150 mg/kg. b.w Group IV rats
Urea	29±2	49.33±5.13	75.67±6.02	86
Creatinine	0.8±0.10	1.38±0.28	1.76±0.06	4.3
Uric acid	3.2±0.36	4.76±1.25	8.13±0.35	8.9
Total protein	6.3±0.20	5.56±0.30	4.36±0.40	2.4
Bilirubin	0.75±0.05	1.32±0.16	2.56±0.40	3.1
Albumin	4.55±0.47	3.47±0.34	2.42±0.09	2
Cholesterol	85±7.93	208.67±8.02	243.67±5.68	279
Triglycerides	85.67±7.50	166±7.55	199.67±6.11	327
HDL	40.33±4.50	29.67±1.52	21±1	14
LDL	27.53±2.80	145.8±7.91	86.06±3.41	199.6
VLDL	17.13±1.50	33.36±1.50	39.93±1.22	65.4
ALT	34±2	51.67±4.04	72.33±5.13	88
AST	27.67±1.52	58±3	82±2.64	95
ALP	76±17.34	139±7.55	152±6.55	184

Mean, standard deviation and one-way ANOVA was done to know the significance. Values were expressed as means±SD. Using one-way ANOVA, the results were significant at 0.001. SD: Standard deviation, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, ALT: Alanine transaminase, AST: Aminotransferase, ALP: Alkaline phosphatase, b.w: Body weight

Table 3: Comparison of blood glucose between four groups

Groups	Blood glucose (mg/dl)				
	0 day	3rd day	1st week	2nd week	4th week
Group I	100.33±4.04	100±4	100±4.35	100.67±3.21	102.67±3.21
Group II	87.67±6.50	142±6	146.33±2.08	146.67±3.21	147.33±3.78
Group III	94.67±8.50	314.33±25.58	316±24.06	317.33±23.45	324.33±24.54
Group IV	100±7	505.33±59.93	508±62.60	491.5±48.79	464

Mean, standard deviation and one-way ANOVA was done to know the significance. Values were expressed as means±SD. Using one-way ANOVA, the results were significant at 0.001. SD: Standard deviation

capillaries were normal. The islets appeared very compact and surrounded by sero-acinar cells. These are stained very deeply with eosin forming the exocrine part of the pancreas, and their nuclei are stained with hematoxylin (Figure 1).

Group II

H and E stained tissue at 400 magnification of 90 mg/kg b.w alloxan-induced rats showed normal architecture of islets of Langerhans. There is no evidence of overt inflammation. The nuclei of the centrally placed cells were normally stained with H and E. No infiltrative changes were observed (Figure 2).

Group III

H and E stained tissue at 400 magnification of 120 mg/kg b.w alloxan-induced rats showed necrosis in the central part of islets of Langerhans. There is a marked decrease in the cells of islets (Figure 3). The islets showed lymphatic infiltration which is a result of inflammation caused to the β-cells (Figure 3). These extensive necrotic changes are accompanied by fibrosis and atrophy.

Group IV

H and E stained tissue at 400 magnifications of 150 mg/kg b.w alloxan-induced rats showed a drastic loss of β-cells (Figure 4). The cells were of relatively smaller size, and reduction in the islet density was observed. Increased eosin staining was a resultant of degenerative changes, followed by fibrosis and atrophy of islets. The islets were seen largely occupied by a uniform eosinophilic material, crumpled in some areas of the sections, and interspersed with few atrophic cells. The exocrine acinar cells were, however, well stained.

DISCUSSION

There is a significant loss of b.w from the day of i.p injection of alloxan at the end of 4 weeks from the original b.w to one-half on an average. There is a sustained hyperglycemia from 102 mg/dl to 464 mg/dl which shows a fourfold increase of blood glucose. The blood urea nitrogen shows progressive azotemia at the end of the 4 weeks. There is a marked decrease in the total protein, albumin, and HDL. There was marked increase in cholesterol, triglycerides, bilirubin, LDL, and VLDL at the end of 4 weeks. The liver enzymes have increased markedly over a period of 28 days. All the parameters mentioned above are compared with control rats.

Injection of alloxan caused an increase of serum cholesterol; the marked hyperlipidemia that characterizes the diabetic state may therefore be a result of the uninhibited actions of lipolytic hormones on the fat depots due to the absence of insulin.[16] Similar increase in the levels of cholesterol was observed in the present study. The present study showed elevation of plasma levels of urea and creatinine which are considered as significant markers of renal dysfunction which is in agreement with the study of Almdal and Vilstrup.[17]

In an earlier work by Jain and Arya[18] advocated that the optimum dosage of alloxan should be arrived at first. This is to avoid erroneous biochemical values in experimental work and reduce the high incidence of mortality in Wistar rats. With a dose of 120 mg/kg b.w of alloxan, there is a marked and stable hyperglycemia of more than 250 mg/dl, and these findings are reflected in a corresponding alteration in the lipid profile. In the present study, stable hyperglycemia was achieved at a dose of 120 mg/kg b.w of alloxan. There is an increase in cholesterol, SGOT and SGPT, and bilirubin. The findings of the present study were similar to those found by Sharma et al.[19]

In the present study, histology of alloxan-induced rats showed necrotic and degenerative changes in the central part of islets of Langerhans. There is a marked shrinkage of the islets. The islets showed lymphatic infiltration which is a result of inflammation caused to the β-cells. These findings are similar to that of previous investigation by Gholamali et al. and Ragavan and Krishnakumari.[20,21]

Biochemical Parameters

The biochemical values obtained were very much significant as shown in Tables 2 and 3.

CONCLUSION

Following the administration of intraperitoneal injection of 90 mg/kg b.w of alloxan, there were no appreciable changes apart from mild lymphocytic infiltration in the islets of the pancreas. With dosages 120 mg/kg b.w and 150 mg/kg b.w, there was a marked loss of β-cells. The islets showed relatively

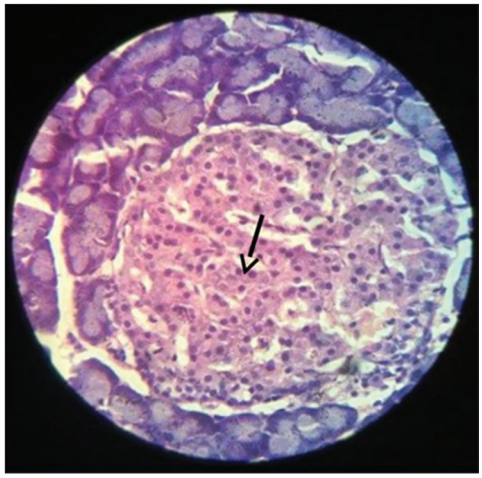

Figure 1: H and E staining of pancreas of control Group at 400 magnifications showing normal architecture of islets of Langerhans and sero-acinar cells

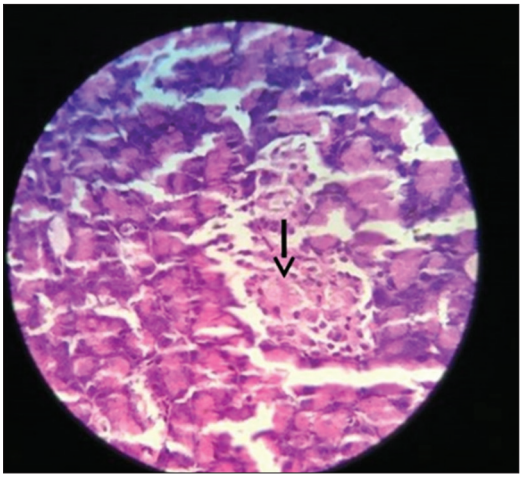

Figure 3: H and E staining of pancreas of Group III at 400 magnifications administered with 120 mg/kg body weight of alloxan showing mildly degenerated and shrunken islets of Langerhans

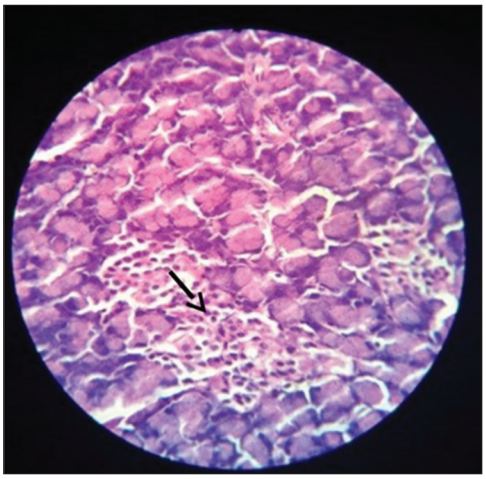

Figure 2: H and E staining of pancreas of Group II at 400 magnifications administered with 90 mg/kg body weight of alloxan showing mildly altered shape of the islets of Langerhans

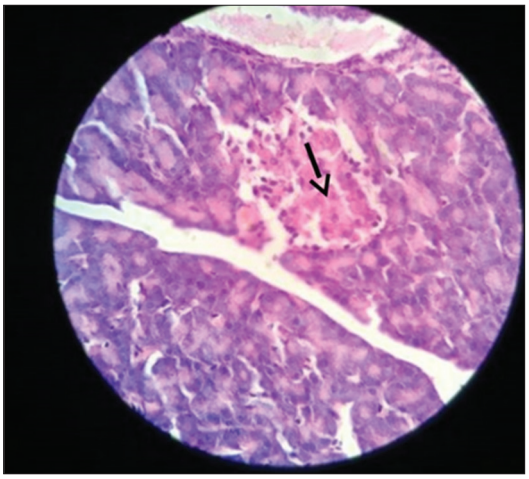

Figure 4: H and E staining of pancreas of Group IV at 400 magnifications administered with 150 mg/kg body weight of alloxan showing necrosis of islets of Langerhans

large spaces indicative of increase in alloxan uptake. Furthermore, the cell outlines could not be discerned when compared to normal control rats. This is because of pyknosis of the β-cell nuclei which undergo karyolysis. In the present study, alloxan given in a dose of 120 mg/kg b.w i.p was found to be optimal for inducing stable and consistent diabetes in Wistar rats and caused a lower incidence of death.

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