Int J Pharm Bio Sci 2015 April; 6(2): (B) 161 - 169

Research Article **Pathology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DIFFERENTIAL EFFECTS OF ALLOXAN ON DIFFERENT BLOOD PARAMETERS IN DIABETEC INDUCED MALE WISTAR RATS

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ABSTRACT

Alloxan is known for chemical induction of Type 1 *Diabetes Mellitus* (DM) through selective damage of β- cells of pancreas, thereby inducing hyperglycemic state in experimental animals. Adult male wistar rats were used as experimental models. Diabetes was induced by injecting alloxan monohydrate at a dose of 60 mg/kg body weight for three days daily. The physical parameters like body weight showed a significant decline whereas there was a rise in food consumption and water intake of the experimental animals. The blood parameters like blood glucose, SGPT, SGOT, and LDH and creatinine levels showed an increase. Blood glucose, LDH and creatinine showed a statistically significant result. However, SGPT and SGOT were statistically insignificant. The study indicates that physical and blood serum parameters might serve as a good indicator for the study of physiology and effects in alloxan induced diabetic rats.

KEYWORDS: **Alloxan , *Diabetes Mellitus*, Hyperglycemia, Blood parameters**

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INTRODUCTION

Diabetes Mellitus has been considered as one of the major health concerns all around the world today.[1-2]It is the most common metabolic disorder characterized by hyperglycemia. It is associated with long term micro vascular and neurologic complications affecting eyes, kidneys, heart, nerves, which are considered the cause of morbidity and mortality in patients with diabetes. [3-4] Experimental animal models are one of the best strategies for the understanding of pathophysiology of any disease in order to design and develop the drugs for its treatment.[5-6-]Several methods[3,7] have been used to induce diabetes mellitus in laboratory animals.The commonly used animal models of diabetes include spontaneouus model, genetic model and chemical induced model. One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan.[7]Alloxan is a urea derivative which causes selective necrosis of the β- cells of pancreatic islets. In addition, it has been widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used.[7,8] A lot of research is still needed to investigate the role of Alloxan and correlation between diabetic signs involving blood serum parameters. The present study was designed to study the effect on bloodserum parameters including food and water consumption and changes in body weight in wistar rats brought about by a single dose of intraperitoneal injection of Alloxan .

MATERIALS AND METHODS

(i) Acclimation of animals in laboratory condition

The consent for working on male wistar rats was taken from the Ethical Committee, Ranchi University, R&D division, Ranchi. Male Wistar albino rats weighing 203.8±2.4 g, six to eight weeks old were used in this study. The animals were procured and raised in the animal house of Dept. of Zoology, Ranchi University as per the norms of Ethical Committee. They were fed the standard diet *ad libitum*. All animals were housed in propylene rat cages with a 12 hour light/dark cycle and free access to rodent chow and water. Experimental animals were divided into two groups (ten rats each) as following:

Group I (control group)

The animals were fed normal food. However, they were injected intraperitoneally (i.p.) for three consecutive days with 100µl saline water from day 1of the experiment.

Group II (diabetic group)

The animals were injected daily with alloxan (60 mg per kg per day) in 0.9% saline water i.p. for three consecutive days. Blood sugar was estimated every three days after the last dose of alloxan to confirm the development of D*iabetes Mellitus* (DM). These animals developed DM in approximately two to three weeks after the first injection of alloxan (mean ± SD) of fasting blood sugar baseline was 295±15 mg dl^{-1}).

(ii) Measurement of blood glucose, body weight, food and water consumption

Blood glucose level and body weight were weekly examined after overnight fasting for 12 hours. Fasting blood glucose level was measured by GOD-POD method. Food and water consumption were measured on a daily basis. Experimental measurements were consecutively performed for 10 weeks.

(iii)Estimation of Glucose[9]

Glucose was estimated by GOD-POD method. One reagent blank and one standard were used for the assay. The samples were mixed well and incubated for 15 minutes at 37 ℃. Absorbance of standard and tests were calculated at 505nm by Systronic's spectrophotometer.

(iv) Estimation of LDH[10]

LDH assay kit uses Lactate dehydrogenase that catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in

absorbance, which is proportional to the LDH activity in the sample.

(v) Estimation of creatinine[11]

Creatinine kit uses the modified Jaffe's method to determine creatinine in serum and urine. Kit contains a picric acid reagent (L1), a buffer reagent (L2), and 2mg/dl of standard creatinine. The absorbance is calculated at 520 nm.

(vi) Estimation of SGPT and SGOT[12]

For both these, mod. IFCC method has been used. There is an enzyme reagent (L1) and one starter reagent (L2).Absorbance was calculated at 340 nm.

(vi) Statistical analysis

Figure data depicted as graphs showing mean values with standard error for each group. Statistical evaluations also included t test and correlation analysis and ANOVA[13].

OBSERVATION

(i) Behavioral changes

2 out of 10 mice treated with Alloxan failed to produce *Diabetes Mellitus* as was studied from its blood glucose parameter. The rest developed the diabetes mellitus as was ensured from the blood glucose parameter. After treatment with alloxan the rats displayed depressed mental status, lethargy, sparse hair con the body and behind the ears. They also developed certain symptoms that were common to diabetic individuals like polyphagia (excessive hunger and feeding), polydipsia (excessive thirst) and polyurea(excessive urination) that have been discussed here in this study.

(ii)Effect on food consumption and water intake

The control mice had a normal consumption and water intake. But after treatment with alloxan, the feeding and water intake increased to a greater extent. It was observed that after nearly 48 hours of alloxan treatment the rats began to consume both food and water to a greater extent as compared to the control. The increase was higher in the first 30 days, after which it maintained a steady state. Both these parameters showed a positive correlation for food consumption and with fasting blood glucose levels in diabetes induced rats.(Fig1 and Fig 2).

Figure 1
Change in consumption of water level

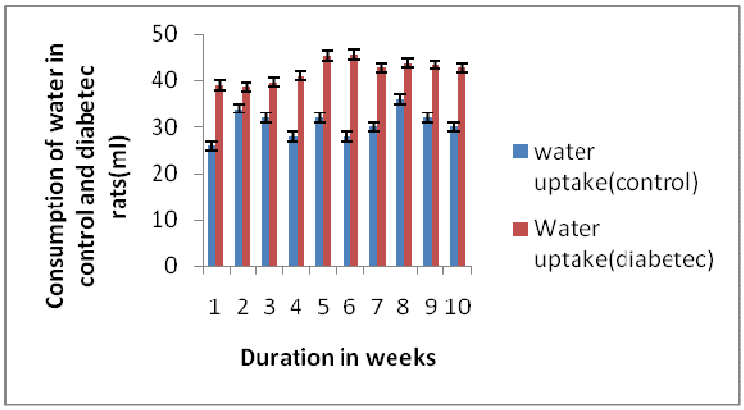
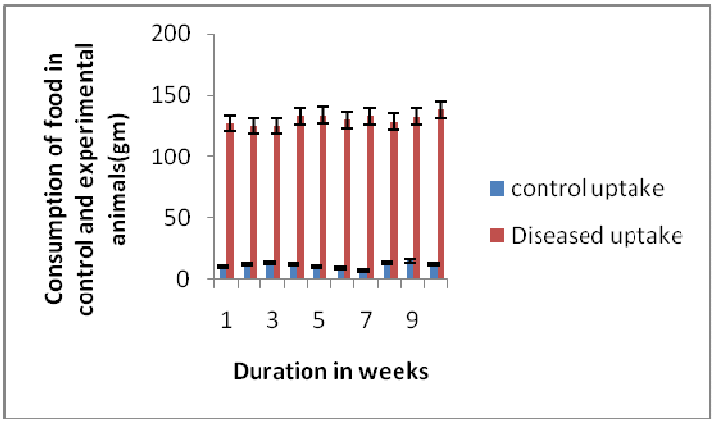


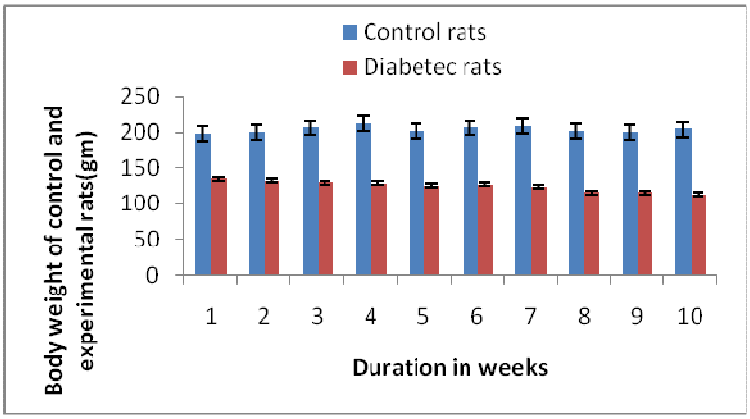
Figure 2
Change in food consumption



(iii)Effect on body weight

The control group showed a slight increase in their body weights, whereas after the treatment with Alloxan the diabetes induced group showed a decline in the body weights just after a few days of treatment (Fig 3).The body weight of diabetic mouse started to decline from the very first week and was significantly ($p<0.0001$) lower than that of control mice from 1 to 10 weeks after alloxan administration. Moreover, correlation analysis showed that the value of body weight was insignificantly correlated with fasting blood glucose level in experimental animals.

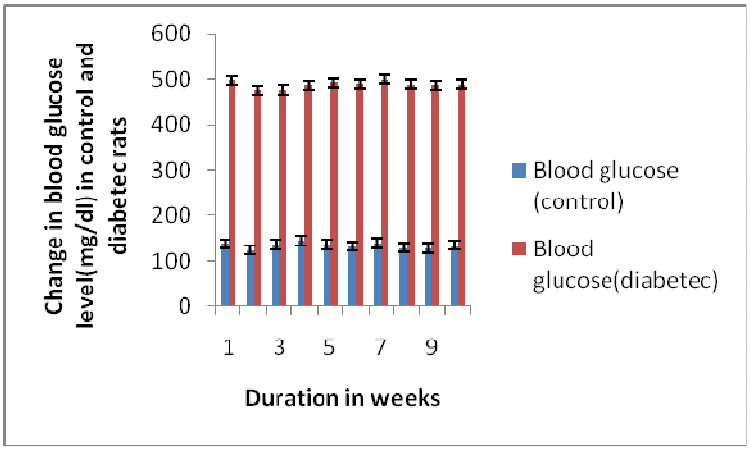
Figure 3
Change in body weight of control and experimental animals on Alloxan treatment



(iv)Changes in the blood glucose level

The control mice did not show any significant change in the amount of blood glucose level. The diabetic rats showed an increase in the blood glucose level after treatment with alloxan in the first week itself. But in the next few weeks the values did not show much difference. But they maintained the hyperglycemic state. (Fig 4)

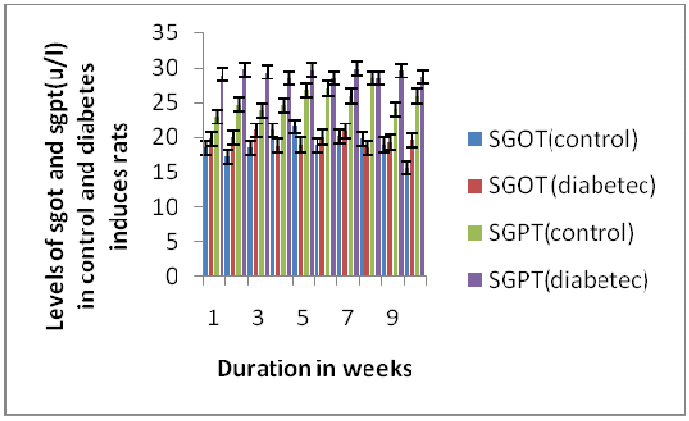
Figure 4
Change in blood glucose level



(v)Changes in SGOT and SGPT levels

SGOT and SGPT did not show a significant change ($p>0.01$). Level of SGOT was highest in the third week and then declined a little. Further, it maintained the above stated condition up till the 10^{th} week. Whereas, SGPT was maximum in the 5^{th} week and then maintained a steady state (Fig 5)

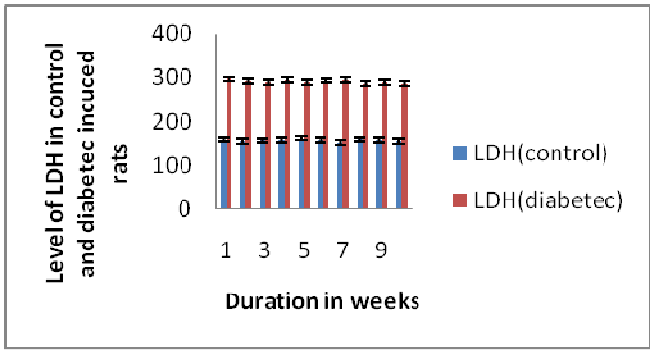
Figure 5
Change in SGOT and SGPT in rats



(vi)Changes in LDH

LDH was much higher in the diabetic rats. This status was maintained right since the first week after the induction of diabetes ($p<0.001$). (Fig 6)

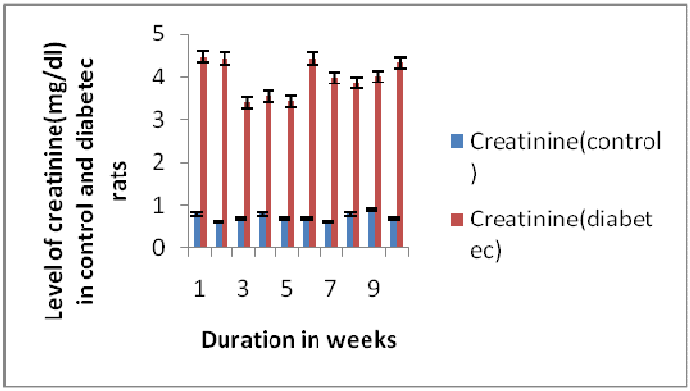
Figure 6
Comparison of LDH in rats



(vii) Changes in level of creatinine

Creatinine level showed an increase after the second week. But after a week did not show much fluctuation ($p<0.001$) (Fig 7)

Figure 7
Comparison of creatinine in rats



RESULTS

The baseline of blood glucose (mg/dl) of control rats was 134.59±3.35 that showed a great hike of 262% being 488.35±3.86 in rats treated with alloxan. An increase in water and food uptake was also noticed in the experimental group. The increase in water uptake (ml) was slight that ranged from 30.8±1.69 in control and 42.22±1.5 in experimental animals that was 37% higher in treated groups. But food consumption (gm) behavior increased to a greater extent from 11.3±1.3 in control to 130±2.47 in alloxan treated group that was nearly an increase of eleven times. A loss in body weight was observed in case of treated animals that was 124.67±4.33 that depreciated by nearly 63% in treated groups as compared to the baseline value of 203.8±2.4.Although there was a slight change in the levels of SGPT (u/l) and SGOT (u/l), there was a substantial rise in the LDH of diabetic rats as compared to the control group. A minimal depreciation of 3.43% was observed in the level of SGOT that was 18.978±0.91 in control animals and 19.63±0.46 in experimental rats. Level of SGPT was 25.39±0.99 in control that increased by 14.76% being 29.14±0.35 in Alloxan treated groups. On the other hand LDH for control animals was 158±1.52 that increased to 291.25±2.28 in treated animals showing 84.33% hike in the treated values. There was a hike in treated group by nearly five times in the case of creatinine assay. The baseline value was 0.73±0,05 whereas that of the treated group was 3..982±0.24 in case of creatinine.

Table 1
The ANOVA result of different parameters of control and treated groups assayed at 95% confidence level

Parameters	*F*	*F crit*
Blood glucose	0.612*	0.314
Creatinine	0.05**	0.314
SGPT	9.083**	3.178
SGOT	4.376*	3.178
LDH	0.578*	0.314

p<0.05; **p<0.01

DISCUSSION

As it has been widely accepted that alloxan selectively destroys the insulin-producing beta-cells found in the pancreas, hence it is used to induce diabetes in laboratory animals. The toxic actions of alloxan on pancreatic beta cells involve oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis.[14-16] The underlying mechanism involves the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism of the pancreatic beta-cells.[17-18] The drug has been noted to exert its diabetogenic action when administered parenterally, i.e., intravenously, intraperitoneally or subcutaneously. Furthermore, the dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status.[19]The present study was an effort to study the mode of inducing diabetes in wistar rats using alloxan with a small dose. It was found that blood glucose increased just after few days and maintained the hyperglycemic condition till the tenth week. The results, evidently, leads to substantiate the induction of diabetes. Further, it was noticed that there was a loss in weight, associated with polyphagia and polydipsia. According to the American Diabetec Association, hyperglycemia does not allows the glucose from blood to enter the cells. The reason for this can be associated with lack of insulin in the body that could lead to persistence of hunger in spite of taking enough food. Thus, the weight of diabetic rats shows a declining trend whereas there is an increase in the food uptake of these rats. The positive correlation between blood glucose and water consumption can be an aftermath of diabetes in diseased rats. The animals consumed more water than the normal rats and even urinated more than the control rats. A probable reason for this could be an attempt made by the kidney to flush the blood sugar from the body thereby depleting water levels in the body. This must be triggering the mechanism of dehydration in cells that finally manifests itself as condition called polydipsia.The present study showed there was very slight change in the levels of SGPT and SGOT in the control as well as diabetic rats. Moreover both these parameters indicated negative correlation with the level of blood glucose. SGOT is present in certain tissues like heart, liver, muscle, brain and kidney. It gets released in the blood stream when any of these tissues get damaged. Hence, it can be said that they are not a perfect indicator of liver damage as it can get elevated in any case of damage to the tissues mentioned .Whereas SGPT is present in large concentrations in the liver. In any case of liver damage its level rises in the blood stream. But in the present study, however, it is not discernible. Moreover, even in case of rise of these two enzymes the liver may keep functioning normally. Thus, it can be said that the damage in liver due to the administration of Alloxan is minimal which does not lead to any liver dysfunction. As in case of creatinine the range of creatinine in normal rats is 0.2-0.8 mg/dl (according to Rats Biochemical Reference Ranges). The level of creatinine is maintained by kidney. If the kidney becomes impaired for any reason, the level of creatinine increases in the blood. This indicates malfunctioning or damage in the kidney. In the present experiment it was found that there is a positive correlation between blood glucose and creatinine level of treated rats .So, its possible that the dose of Alloxan may account for poor clearance of creatinine by the kidney. The

levels of plasma ldh levels were also found to increase in the experimental groups. Celik and Yegin[20] also noticed this increase as important evidence that supports diagnosis of diabetes on treatment with Alloxan. Some investigators suggests that changes in enzyme activity can result from influence of insulin on liver and muscle tissue.[21] Moreover the results of ANOVA also ascertain that the values are significant (Table1).It was seen $F_{observed}$ is larger than $F_{critical}$ from the table, hence we can reject the null hypothesis and state that the variance between groups is not due to random chance, but rather due to the influence of a tested factor at 95% confidence level. *Diabetes Mellitus* is a major endocrine disorder affecting nearly 10% of the population all over the world.[22]So the hyperglycemia gives a clear cut implication that administration of glucose does has a significant effect on pancreas that leads to diabetogenic changes in the body of experimental rats. The level of SGPT and SGOT suggests that liver may not be the target area of Alloxan. The mild change in liver enzymes is not suggestive of any damage in liver. But kidney noticeably gets affected to an extent as seen with the increased levels of creatinine in the body of diseased rats.

CONCLUSION

The present study is, thus, an effort to illustrate that Alloxan induced diabetes is one of the reliable methods of diabetes induction in experimental animals. It is associated with low mortality and morbidity of experimental animals as compared to the genetic or surgical removal of pancreas methodologies. Moreover, duration of ten weeks can be a substantial period of studying the effects of Alloxan on experimental animals. It also explains that certain increase in some enzymes could be due to malfunctioning of some organs as a result of non absorbance of glucose by the body cells. Alloxan induced diabetes can be a better way of assessing diabetes and certain other parameters related with it.

ACKNOWLEDGEMENT

The Financial assistance provided by CSIR-UGC for this work as a part of Minor Research Project {Research Project no. F.PSJ-006/12-13(ERO)} is greatly acknowledged.

CONFLICT OF INTEREST

There is no conflict of interest in the work done.

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