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Re-Evaluating Antidiabetic Effect of Pioglitazone in Alloxan Induced Diabetic Animal Model.

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ABSTRACT

The study includes the pharmacological screening on Pioglitazone, which is an effective and frequently prescribed treatment for type 2 diabetes. An in-vivo study included three animal models with six mice in each, one was control or untreated and other two groups were alloxan and one group drug treated to compare the pharmacological response of pioglitazone against diabetic. Alloxan was used to induce diabetes in animals. Alloxan, a β -cytotoxin, chemically called mesoxalylurea, mesoxalylcarbamide, and 2, 4, 5, 6-tetra-oxohexahye-4-pyrimide or pyrimidine tetrone has been extensively used for in vivo induction of 'chemical diabetes' in animals. Administration of Pioglitazone reduced serum glucose level in alloxan (70 mg/kg) induced diabetic mice as compared to vehicle.

INTRODUCTION

Diabetes Mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence in 1985 of 30 million, in 1998 of 143 million, in 2000 of 150 million people, and is expected to increase to 220 million people by 2010 and recently estimated project that the number of patients diagnosed with type 2 diabetes will be more than double to 300 million before 2025^[1]. Type 1 diabetes describes as Insulin dependent diabetes mellitus (IDDM) or Juvenile onset diabetes mellitus and Type 2 diabetes describes as Non Insulin dependent diabetes mellitus (NIDDM) or Adult onset diabetes mellitus. Antidiabetic drugs have ability to decrease serum glucose level in diabetic animals with different mechanisms. There are various models for the study of this property of drugs in different laboratory animals. The thiazolidinediones (TZDs, or glitazones) class, which currently includes rosiglitazone and pioglitazone, are effective and frequently prescribed treatments for type 2 diabetes that complement existing treatment approaches and form an important part of treatment algorithms. In the decade since their introduction, the prevalence of obesity, diabetes, and the metabolic syndrome has increased exponentially^[2,3].

MATERIALS AND METHOD

Alloxan Monohydrate

Alloxan was used to induce diabetes in animals. Alloxan, a β -cytotoxin, chemically called mesoxalylurea, mesoxalylcarbamide, and 2, 4, 5, 6-tetra-oxohexahye-4-pyrimide or pyrimidine tetrone has been extensively used for in vivo induction of 'chemical diabetes' in animals. It causes diabetes in animals by its ability to destroy the insulin-producing β cells of the pancreas. Alloxan and N substituted alloxan derivatives were selectively toxic to pancreatic β cells, with other endocrine cells and exocrine parenchymal cells being well preserved, even at high concentration^[4,5].

Preparation of 70 mg/kg of Alloxan monohydrate solution

Weigh 0.7 gm of alloxan monohydrate accurately and transfer to 100 ml of water for Injection and dissolve alloxan in it. We got the concentration of solution 7 mg/ml.

Pioglitazone

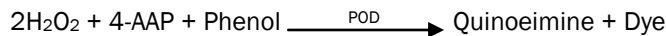
Pioglitazone is a thiazolidinediones (TZDs) that act primarily by decreasing insulin resistance. Their mechanism of action for improving insulin sensitivity is not yet fully understood. They are selective agonists for peroxisome proliferator-activated receptor gamma (PPAR γ). Activation of PPAR γ receptors results in increased glucose transport into cells in adipose tissue, skeletal muscle, and liver [6].

Animal Model

The ethical approval has been taken before choosing animal model. Swiss Albino mice of either sex weighing between 29-36 gm \pm 5 gm were used in present antidiabetic screening. The animals were housed in polypropylene cages. The bedding material of cages was changed regularly. The temperature of the experimental room was maintained constant at 25°C and lightening was kept artificial. The sequence being 12 hrs light and 12 hrs dark. Conventional laboratory diets and water were provided *ad-libitum*.

Glucose Estimation Kit (GOD-POD Method)

The reagents are used for the quantitative determination of Glucose in serum or plasma. Serum Glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4 amino- antipyrene(4-AAP) and phenol in the presence of peroxidase(POD) to yield a red quinoneimine dye that is measured at 505nm. The absorbance at 505 nm is proportional to concentration of glucose in the sample.



Absorbance of the colour solution is directly proportional to the glucose concentration, when measured at 505nm [7].

In-vivo Animal Study

Removal of blood by Retro Orbital Puncture (ROP) method

Mice were divided in to sixteen groups of six each and marked with picric acid.[8] They were fasted for 18 hrs and water was given *ad libitum*. After 18 hrs the fasting blood samples were collected by retro orbital puncture (ROP). Collect the blood sample flowing from the capillary into the bottle containing anticoagulant solution about 0.5-1 ml of blood.

Preparation of testing by Accurex Glucose Stat colorimetric test God Pod method

The initial fasting Serum Glucose was estimated by enzymatic colorimetric method. As per this withdraw blood from the animal (Mice) in 1.5 ml micro-centrifuging tubes and separate the plasma within 30 minutes by centrifugation at speed of 2800 RPM for 15 min and transfer 10 μ l of sample to the working reagent tubes, mix and incubate for 15 min at 37°C or 30 min. at room temperature [9]. Measure the absorbance of sample (A_s) or standard (A_{std}) prepared against blank at 505 nm [7,10]. Zero spectrophotometer against water as blank.

Determine the result as under:

$$A_s \times \text{Conc. of standard} / A_{\text{std}}$$

Glucose (mg/dl) =

To convert result to mmol/l multiply mg/dl by 10.
To convert to mg/l and divide by 180 (mol. wt. of glucose)

Selection of Animal for induction of Alloxan

The animal showing very high > 150 mg/dl or low < 75 mg/dl Serum glucose level (SG) were discarded while the animals showing optimum SG 80 - 120 mg/dl were selected for study and injected with alloxan (70 mg / kg) i.v.

Administration of Alloxan monohydrate solution

10 mg/ml Alloxan solution is administered through intravenous route in mice with dose of 100mg/kg, depends on the body wt. of animals for induction of diabetic.

Selection of Animal for Study

After 48 hrs of alloxan injection the blood was removed by retro orbital puncture and SG was estimated by GOD/POD method as done for earlier. The animals showed SG above 200 mg/dl were selected for study. Total eighteen animals in three groups i.e. six animals in each groups have been selected for the study and they were grouped as follows

Group I	Vehicle (distilled water; 10 ml/kg, p.o.)
Group II	Alloxan (70 mg/kg, i.v.)
Group III	Pioglitazone (30 mg/kg)

Group I, included six mice which were not treated with either Alloxan or Pioglitazone.

Group II, included six mice which were treated with only Alloxan.

Group III, included six mice which were treated with both Alloxan and Pioglitazone.

Oral Drug Administration

Use a hypodermic needle, bent by 30° around 1cm distance from tip, gauge blunted at the tip to avoid any injury to the inner surface of mouth and oesophagus. This is the feeding cannula. Attach the cannula to a calibrated syringe. Withdraw required amount of drug solution/suspension in the syringe. Remove air bubbles and readjust the volume of solution/suspension. Hold the mice by the nope by one hand thus making the animal to open its mouth. Insert the feeding cannula through the intra-dental space and gently push into oesophagus. Adjust the desired position of cannula and gently push the piston of syringe to administer the accurate volume of drug solution. If by chance the drug enters into the lungs then hold the animal by tail and suspend in air. Give light pat on its back and try to expel the drug from lung.

Preparations and Administration of Doses

The test compounds were administered orally. The doses were given to the mice in stepwise procedure using fixed doses of 30-mg/kg body weight.^[11,12] Animals were fasted for 18 hours prior to dosing with free access to water. Food was given to the mice 3 to 4hr after administering the test materials ^[11,13].

Collection of Blood Sample

Blood samples were removed from all animals at 2, 4, 6 and 24 h by retro orbital puncture method and SG was estimated by GOD/POD method.

Percentage change in SG, average and SD were calculated and tabulated.

$$\% \text{ Decrease in blood glucose} = \frac{\text{BSL of control} - \text{BSL of drug}}{\text{BSL of control}} \times 100$$

Statistical Analysis

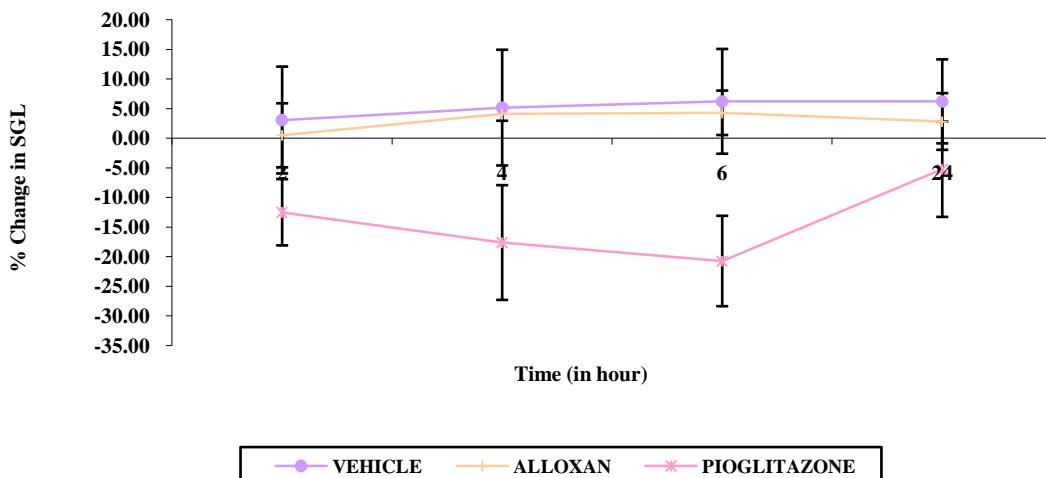
The antidiabetic activity was assessed by alloxan induced antidiabetic method using UV-Visible spectrophotometer for determination of BSL at different time intervals. The data obtained were analyzed by one-way ANOVA followed by Dunnet-t test. The results were expressed as mean, \pm standard error of mean (SEM) for each group, $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Administration of Pioglitazone reduced serum glucose level in alloxan (70 mg/kg) induced diabetic mice as compared to vehicle (distilled water, 10 ml/kg, p.o.) treated group (Table 1and Fig 1).

Table: 1 Hypoglycemic Activity of Pioglitazone in alloxan induced diabetic mice

Drug	Percentage change in SG level at given time in each groups			
	2 h	4 h	6 h	24 h
Vehicle	+3.06±9.02	+5.15±9.77	+6.21±8.85	+6.21±7.09
Alloxan	+0.48±5.41	+4.09±1.16	+4.28±3.75	+2.81±4.78
Pioglitazone	-12.52±5.59	-17.63±9.69	-20.75±7.63	-5.25±8.05

Figure 1 Hypoglycemic Activity of Pioglitazone in alloxan induced diabetic mice

CONCLUSION

Pioglitazone was tested for anti-hyperglycemic activity by alloxan induced diabetic mice method. Activity was presented in the form of percent decrease in blood glucose level. Activity was compared with vehicle treated group and the statistical significance of the data was analyzed by ANOVA followed by one-way Dunnet “t” test. In conclusion, Pioglitazone was a potent alternative to some of the related antidiabetic agents.

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