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DEVELOPMENT OF CHICK EMBRYO DIABETES MODEL FOR SCREENING OF ANTIDIABETIC DRUGS

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ABSTRACT The aim of the present study was to investigate whether young chickens with diabetes could be developed by treating the chick during embryonic stage with streptozotocin (STZ) or alloxan. The model was validated for testing antihyperglycemic activity using a standard drug, glipizide. Hatched eggs were divided in to three groups. On the day 14 of incubation, holes were made on the shells of the eggs by using driller, and then were injected with STZ [0.3mg/kg] and alloxan.Control group kept with out any treatment [n=8 in each group]. The holes were closed using tape and the eggs were incubated for another 7days (up 21st day). Chicks that would came out were kept in specially made cages .After two weeks ,blood glucose levels were estimated using chemstrip method using Glucometer.Hyperglycemic chicks were separated and treated with standard drug glipizide[0.5mg/kg],and the blood glucose levels were estimated at different time intervals [30, 60, 120 min].The results were analyzed statistically by ANOVA. The levels of blood glucose significantly [STZ& Alloxan: **P <0.01] increased in STZ/alloxan treated groups as compared to the control and there was a significant [STZ: *P <0.05; Alloxan **P<0.01] decrease after treating with standard drug within 30-60min. The results of the present study indicate that STZ, alloxan-if administered during embryonic stage would result in a stable diabetic chicks after hatching and this model can be used to screen drugs for anti-diabetic activity.

KEYWORDS : Chick diabetic model, chick embryo, antidiabetic, screening, antihyperglycemic, hypoglycemic.

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by disordered glucose metabolism and abnormally high blood sugar (hypergl ycemia) resulting from low levels of the insulin with or without abnormal resistance to insulin's effects (Rang *et al*., 2007). New drugs may be discovered from a variety of natural sources or created synthetically in the laboratory. They may be found quite by accident or as a result of many years of tireless pursuit. Recently, most of the synthetic compounds and natural plants are screened for anti-hyperglycemic activity (Reddy *et al*., 2010). In an attempt to reduce the number of mammals used in drug research, we have been examining the use of chicks and found that they may be superior for predicting the effect of dugs. To screen compounds for antidiabetic activity, we have to use animals like rats to induce diabetic by administration of either Alloxan or Streptozotocin (Vogels *et al*., 2002). In order to reduce the time and cost of the experiments, we have planned to use chicks to develop diabetic chicks or model for the screening. It is also reported that administration of alloxan or STZ is unable to produce diabetes in chicks (Danby *et al*., 1982). So we thought to develop and produce diabetic chicks by administration of alloxan or STZ to the fertile eggs. So far no reports are available on chicks as a diabetic animal model to screen compounds for antidiabetic activity. The main objective of this study was to develop and validate diabetic chick model for screening of anti diabetic activity.

MATREALS AND METHODS

Materials

Streptozotocin and Alloxan were procured from Sigma, St.Louis, MO. USA. Glipizide was a kind gift sample of Dr Reddy s laboratory, Hyderabad. Fertilized chicken eggs were supplied by Thirumala Hatcheries, Hanamkonda. Incubator is belongs to KV technology Corporation, Hyderabad.

Methods

Collection of fertile eggs: Fertile chicken eggs (65-70 grams) were obtained from Thirumala Hatcheries, Hanamkonda, Warangal, India. They were incubated in a specialized Hatching incubator where temperature (99^{0}F) and humidity (87%) were maintained.

***Induction of diabetes*:** Alloxan and STZ were separately used to induce diabetes in chicks. For the induction of diabetes, we have used 0.6, 0.9 and 1.5mg/egg doses of both.

Procedure: Fertile eggs of white leghorn chicks were incubated for 14 days (99^{0}F.87% humidity). On the 14th day of incubation, small holes were made on the shells using driller, and then the diabetogenic agents like alloxan or STZ of different concentrations (i.e. 0.6, 0.9 and 1.5mg/50 gr egg) were injected into sac of each egg respectively under sterile conditions. After injection, holes were closed using plaster or tape then eggs were incubated for another 7 days. On the 21st day of incubation, chicks were come out from the eggs. After 2 weeks of their birth, glucose levels were estimated by taking the blood from tip of finger using chem strip method. The chicks having high blood glucose levels more than normal were considered as diabetic chicks.

3) Screening of oral hypoglycemic agents using chicks as a model.

The alloxan induced diabetic chicks (fasting) were divided into three groups of six in each and treatment was done as follows.

Groups I: Served as normal control and treated with normal saline

Group II: Served as Alloxan induced diabetic control solvent

Group III: Treated with single dose of glipizide 0.5mg/kg

The STZ induced diabetic chicks (fasting) also divided into three groups of six in each and treatment was done as follows.

Groups I: Served as normal control and treated with normal saline

Group II: Served as STZ induced diabetic control solvent

Group III: Treated with single dose of glipizide 0.5mg/kg

The blood samples were collected from fingertip at different time intervals (0, 30, 60 and120 min) and fasting blood glucose levels were estimated using chem Strip method.

RESULTS

In the present study, we have used three different doses [0.6, 0.9 and 1.5mg/egg] of alloxan and STZ to produce diabetic chicks and the results were showed in table 1&2. Injection of alloxan at these three doses into the sac of fertile eggs at 14th day of incubation resulted in the production of diabetic chicks. The blood glucose levels of diabetic chicks treated with different selected doses [0.6, 0.9 and 1.5mg/egg]

were significantly higher (**P<0.001) than those of normal chicks.

Table 1 Dose titration of Alloxan in chick embryos to induce diabetes in chicks

Control	0.6mg/egg	0.9mg/egg	1.5mg/egg
238.5±3.9	289.83±5.1**	339.3±5.7**	357.17±4.6**

Data expressed as Mean±SEM [n=6]. **P<0.001

Table 2 shows the mean fasting blood sugar levels of both STZ [0.3mg/kg](5) and alloxan [0.9 mg/kg] treated and control groups. It also showed that STZ at 0.3mg/kg was producing the diabetic chicks having high blood glucose levels than those of alloxan at 0.9mg/kg.

Table 2 Induction of hyperglycemia in chicks using STZ and Alloxan

Control	STZ (0.3mg/egg)	Alloxan (0.9mg/egg)
242±4.1	499.29±9.6**	452.71±10.1**

Data expressed as Mean±SEM [n=6]. **P<0.001

The effect of glipizide (0.5mg/kg) on the blood glucose levels of diabetic chicks at different intervals were showed in table 3. The significant (p<0.05) glucose levels were reduced with glipizide after 30 minutes of its administration, indicating its antihyperglycemic activity in diabetic chicks.

Table 3 Effect of blood glucose levels in Alloxan and STZ induced diabetic chicks after treating with glipizide [0.5 mg/kg]

	Alloxan	STZ
Weight (g)	69.29±1.8	69.71±2.1
0min	452.71±10.1	499.29±9.65
30 min	268.43±9.4*	285±10*
60 min	130.57±6**	211.71±6**
120 min	194.29±6.33**	272.14±5.3**

Data expressed as Mean±SEM; [n=6].*P<0.05, **P<0.001.

DISCUSSION

In the present study, we have developed an experimental chick model to screen the compounds for antihyperglycemic activity. We have used alloxan and STZ to induce diabetic chicks by injecting them into the fertile egg sac at 14th day of incubation and at 21st day of incubation, we got the chicks those blood glucose levels were significantly higher (p<0.05) than those of normal (238.5±3.9 mg/dl), hence we categorized as diabetic chicks. Then we have titrated the dose of alloxan as 0.9mg/kg and STZ as 0.3mg/kg to induce diabetes in chicks. The antidiabetic activity of the glipizide also proved in diabetic chicks. This indicated the successful induction and development of diabetic chick model for the screening of compounds for the antidiabetic activity.

Danby et al., (1982) tried to calculate medium lethal dose (MLD) for Alloxan & STZ by injecting systematically into chicks but failed to calculate dose of Alloxan & STZ to cause diabetes in chicks. Then we have planned to produce diabetic eggs by injecting alloxan or STZ into fertile eggs and successfully produced the diabetic chicks. The dose of alloxan used to induce hyperglycemia in chicks (0.9mg/egg i.e. 15mg/kg) was lower than that of rats (120mg/kg in rats). Hence this model can be used as an alternative model fore the rat or mouse models.

Glipizide significantly reduced the blood sugar levels in both alooxan and STZ treated groups at different time points *i.e.* at 30, 60, 120 min in diabetic chicks. But the glucose levels at 120 min is higher than that of the glucose levels at 60min.This increased blood glucose level after 3hrs indicates short duration of action of glipizide in chicks.

CONCLUSIONS

The following conclusions could be drawn broadly from the results of these investigations.

1. The doses of Alloxan & STZ which were used to induce hyperglycemia in chicks, was found to 0.9 & 0.3mg/egg respectively for 60 grams egg.
2. Chicks will used for screening of anti hyperglycemic agents like glipizide and it was observed that the blood glucose levels of chicks were reduced.

Hence, we conclude that chick embryo diabetes model can be used as novel model for induction of diabetes as well as for screening of oral hypoglycemic agents.

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