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RESEARCH ARTICLE

ANTIDIABETIC ACTIVITY OF 2-AMINO-(5-FLUORO-2-OXOINDOLIN-3-YLIDENE) BENZOXAZOLE-5-CARBOHYDRAZIDE IN RATS

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ABSTRACT

The present study was designed to evaluate the antidiabetic activity of new indole derivatives i.e 2-Amino-(5-fluoro-2-oxoindolin-3-ylidene) benzoxazole-5-carbohydrazide against alloxan induced diabetic rats. Alloxan (120mg/kg) was used to induce diabetes in rats and the blood glucose levels were estimated by using commercial kit in the market. The test compound was administered to diabetic rats as single dose for one day at a dose of 50 & 100 mg/kg. The compound produced a significant reduction ($p<0.01$) of blood glucose levels at a dose of 50 & 100mg/kg in diabetic rats in a dose dependant manner.

Keywords: Diabetes mellitus, Antihyperglycemic, 2-Amino-(5-fluoro-2-oxoindolin-3-ylidene) benzoxazole-5-carbohydrazide, Alloxan.

INTRODUCTION:

There is a global increase in the prevalence of diabetes mellitus predominantly, related to life styles and the resulting surge in obesity. Diabetes mellitus is one of the most common metabolic disorder with long term macrovascular and microvascular complications includes diabetic nephropathy, neuropathy, and retinopathy that results in significant morbidity and mortality (David *et al*., 2009). This metabolic disorder is seen worldwide and its occurrence is increasing fast in most of the countries (Siddharth, 2001). Diabetes mellitus is characterized by hyperglycaemia, carbohydrate, protein, fat metabolism. Besides hyperglycaemia it is also associated with defect in reactive oxygen species scavenging enzymes (Kesavulu *et al*., 2000). None of the oral synthetic hypoglycemic agents has been successful in maintaining euglycaemia and controlling long-term microvascular and macrovascular complications due to their side effets (Deb L and Dutta , 2006).

The test compound i.e. 2-Amino-(5-fluoro-2-oxoindolin-3-ylidene) benzoxazole-5-carbohydrazide, selected for evaluation of antidiabetic activity is based on its potential antioxidant, anticancer and antimicrobial activities (Rajyalakshmi et al., 2011).

MATERIALS AND METHODS:

Chemicals:

Alloxan monohydrate (Sigma, St Louis, U.S.A), Glibenclamide (Gift sample, Dr. Redyy's, Hyderabad), Methanol (E-Merck, Mumbai,India), Sod CMC (Central drug house, New Delhi)

Test Compound:

The test compound i.e 2-Amino-(5-fluoro-2-oxoindolin-3-ylidene) benzoxazole-5-carbohydrazide is a novel indole or isatin derivative and the synthesis and biological activities of the compound was previously reported (Rajyalakshmi et al., 2011). The test suspension was prepared using 0.5% Sod CMC

Animals:

Male albino Wistar rats weighing 150-180g (4-8weeks) used in this study were procured from Mahaveera Enterprises, Hyderabad and were housed in polypropylene cages in a room of temperature 23± 2^0c and relative humidity 50% with 12:12hr light:dark cycle. Animals were acclimatized to this environment throughout the period of experimental study. They were with standard food and water *ad libitum*

Acute toxicity study:

Acute toxicity studies were performed by using male Wistar rats. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. MEHB was administred orally using various doses upto 1500mg/kg and observed for the mortality and behavioral changes. (OECD, 2000).

Evaluation of anti-diabetic activity in rat model:

Alloxan monohydrate (2, 4, 5, 6,-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) at dose of 120mg/kg

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(intraperitoneally in normal saline) was used to induce Diabetes mellitus in rats (Ragavan B and Krishnakumari, 2006). After one hour of alloxan administration the animals were given feed *ad libitum* and 5% dextrose solution were also given in feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation for about 48hrs. The animals were kept fasting overnight and blood glucose levels were estimated before and after 72hrs of alloxan treatment. Animals showing blood glucose levels of >200mg/dl is considered as diabetic and were used for study.

Study Design:

All the fasting (for 12 hr) diabetic rats were divided into four groups of each six (n=6).

Group I: Normal rats served as normal control and treated with (1ml/kg, p.o) of 0.5% Sod CMC.

Group II: Served as diabetic/disease control and received 0.5% Sod CMC (1ml/kg, p.o)

Group III: Diabetic Rats treated with test compound at a dose of 50mg/kg, (p.o)

Group IV: Diabetic Rats treated with test compound at a dose of 100mg/kg, (p.o)

Group V: Diabetic Rats treated with glibenclamide (2.5mg/kg,p.o) & served as standard group (Dash *et al*., 2008).

The treatment was given for 1 day and blood samples were collected at different intervals

Collection of blood samples:

Blood samples were collected from all the groups of animals at 0, 1,3,5,7 hr intervals through puncture of retro orbital plexus and were centrifuged at 10000rpm for 10 minutes. Serum was separated and stored at -20^{0}C and then used for estimating Blood Glucose levels (Span Diagnostics, Surat, India).

***In vitro* glucose uptake method using rat hemidiaphragm:**

In vitro glucose uptake by the rat hemidiaphragm models has been used to assess the *In vitro* antidiabetic activity of test compound (Chattopadhyay *et al*., 1992). In the present study, we have used this *in vitro* study of glucose uptake by rat hemidiaphragm method to evaluate the antidiabetic activity of 2-Amino-(5-fluoro-2-oxoindolin-3-ylidene) benzoxazole-5-carbohydrazide

Study design:

Four sets of experiments were performed. The animals were killed by decapitation and diaphragms were exposed to

Group I: 2 ml of Tyrode solution with glucose (2%) only and served as control.

Group II: 2 ml of Tyrode solution with glucose (2%) + 0.5 ml of test (50 mg/ml)

Group III: 2 ml of Tyrode solution with glucose (2%) +0.5 ml of test (100 mg/ml)

Group IV: 2 ml of Tyrode solution with glucose (2%) +/ 0.62 ml of insulin (0.4 Unit/ml)

Group V: 2ml Tyrode solution with glucose (2%) +/ 0.62 ml of insulin (0.4 Unit/ml) +0.5 ml of test (100 mg/ml) (Walaas and Walaas, 1952; Chattopadhyay *et al*., 1992).

Statistical analysis:

All the experimental values were expressed as mean ± SD (n=6). One-way ANOVA and Dunnett's test were used to compare means from the control group and each of the groups exposed to toxicant and test compound. The statistical significance was judged at the 0.05 probability level.

RESULTS:

Acute toxicity study:

The LD_{50} dose of the test compound was found at 1500 mg/kg b.w. p.o. and 50 mg/kg and 100 mg/kg were used as the test doses for the evaluation in the present study.

Effect of test compound on Blood Glucose Levels in Alloxan induced diabetic rats:

The effect of MEHB on blood glucose levels were shown in Table 1. Alloxan monohydrate adminsitartion at a dose of 120mg/kg to rats successfully produced diabetes by elevating blood glucose levels grater than 200mg/dl. Administartion of test compound at a doses of 50 & 100mg/kg to diabetes rats produced a significant reduction in the blood glucose levels in a dose dependant manner ($p<0.01$) (table 1).

Effect of test compound on Blood Glucose Levels (mg/dl) in rat hemi diaphragm method:

Similar to the Insulin, in presence of test compound at doses of 50 & 100mg/kg, it was observed that there is enhancement of the glucose uptake by the hemi diaphragm, (skeletal muscle). The statistically significant difference ($p<0.01$) was observed in the utilization of glucose uptake in presence of test compound by the hemi diaphragm and was observed as time dependant. This indicates the mechanism of action of MEHB for its antidiabetic activity (table 2).

Table 1: Effect of Test compound on serum glucose levels in diabetic rats

Group/time	0hr	1hr	3hr	5hr	7hr
Normal control	85.9±8.2	86.2±7.8	90.2±8.4	88.8±7.5	90.5±8.7
Diabetic control	311.3±26.7	309.5±31.8	315.4±30.0	322.4±27.3	325.8±27.1
Diabetic+Test (50mg/kg)	285.6±24.2	276.2±25.3*	241.7±21.6**	225.2±18.9**	210.0±17.6**
Diabetic+ Test (100mg/kg)	297.5±22.4	274.2±22.1**	221.6±18.4**	198.2±14.8**	180.7±15.1**
Diabetic+Glibenclamide (2.5mg/kg)	292.9±20.5	256.3±17.5**	197.9±17.3**	144.1±11.4**	135.1±11.2**

All the values of Mean±SD; n=6; *p<0.01, **p<0.001 vs diabetic control,

Table 2: Mean ± SD of Glucose Levels (mg/dl) in rat hemi- diaphragm method

Group/time	0hr	1hr	3hr
Normal control	630.8±20.7	605.2±19.6	571.1±17.5
Test compound (50mg/ml)	638.1±19.7	601.2±17.8*	562.6±18.4**
Test compound (100mg/ml)	645.2±18.7	592.1±18.3**	548.3±17.3**
Insulin (1 Unit/ml)	678.5±28.7	604.1±18.0**	538.5±15.9**
Test compound (100mg/ml) + Insulin (1 Unit/ml)	690.8±6.7	600.3±22.1**	510.1±19.4**

All the values of Mean±SD; n=6; *p<0.05, **p<0.01 vs control,

DISCUSSION:

Diabetes is a chronic metabolic disorder caused by either insulin deficiency or insulin resistance, which produces inadequate glucose control and leads to acute and chronic complications (Chattopadhay, and Bandyopadhyay, 2005). Glucose control is essential, but this provides only minimal benefit with respect to CHD prevention. An ideal treatmant for diabetes would be a drug that not only controls the glycemic levels but also prevents the development of arteriosclerosis and other complications of diabetes (Halliwell and Gutteridge, 1985).

Alloxan (2, 4, 5, 6,-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative (Lenzen, 2008). Alloxan is toxic glucose analogue, when administered to rats and many other species, which selectively destroys insulin-producing beta cells in the pancreas reaulting in insulin- dependent diabetes mellitus (Alloxan Diabetes) with characteristics similar to type 1 diabetes in humans (Lenzen, 2008).

The present study results suggest that the test compound exhibited significant antihyperglycemic activity in alloxan induced diabetic rats. Fasting blood glucose level in diabetic rats is an important basal parameter for monitoring diabetes (Rajkumar *et al*., 2005) and it has shown that the test compound causes the antihyperglycemic effect by reducing ($p<0.05$) the fasting blood glucose level in a dose dependant manner.

The significant decrease in the levels of fasting blood glucose in diabetic rats treated with the test compound may be by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilisation of glucose. In the present *in vitro* study of glucose uptake method results indicate that test compound significantly increase the glucose uptake and results were similar to that of insulin. These findings suggest that the test compound might have direct metformin like activity which enhances the peripheral utilization of glucose and have extra pancreatic effect. The uptake of glucose by the skeletal muscles has been potentiated in presence of insulin and test compound, which may be responsible for antdiabetic activity.

CONCLUSION:

In conclusion, the present study results indicated that test compound was endowed with antidiabetic activity.

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