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Research Article

Antidiabetic effect of 2 nitro benzimidazole in alloxan induced diabetic rats

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ABSTRACT

Background: The objective of study was to evaluate the antidiabetic activity of 2 nitro benzimidazole in alloxan induced diabetes in rats.
Methods: Alloxan induced diabetic rats (n = 6) were administered 2 nitro benzimidazole (15 mg/kg, p.o.) or vehicle (gum acacia solution) or standard drug glibenclamide (10 mg/kg) for 7days. Blood samples were collected from the tail vein and blood glucose levels were estimated on days 1, and 7 at 0, 2, 4 and 6 hr after administration of drug by a glucometer.
Results: The test drug 2 nitro benzimidazole induced significant reduction ($P < 0.001$) of blood glucose levels in alloxan induced diabetic rats at 2nd and 4 hr comparable with glibenclamide on the 1 and 7th days. The results were analyzed for statistical significance using one-way ANOVA, followed by Dunnet's test. $P < 0.05$ was considered significant.
Conclusion: 2 nitro benzimidazole exhibited significant antihyperglycemic activity in alloxan-induced diabetic rats comparable to that of the standard drug glibenclamide.

Keywords: Alloxan, Antidiabetic activity, 2 nitro benzimidazole, Glibenclamide

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both.[1] The metabolic dysregulation associated with diabetes mellitus causes secondary pathological changes in multiple organ systems accompanied by micro vascular as well as macro vascular complications.

The two main types of diabetes mellitus are type 1, and type 2. Type 1 diabetes is caused by the autoimmune destruction of the beta cells of the pancreatic islets, whereas type 2 diabetes results from both impaired insulin secretion and resistance to the action of insulin.

Diabetes is the most important non infective epidemic to hit the globe in the present times. By the year 2025, India shall have the maximum number of diabetics in the world making it the "Diabetic capital of the World."[2]

Currently anti diabetic drugs like sulfonylureas, meglitinides, the biguanide metformin, thiazolidinediones, alpha-glucosidase inhibitors, and the oral dipeptidyl-peptidase-4 inhibitor sitagliptin are used in the management of diabetes. The progressive deterioration of diabetes control is such that after 3 years approximately 50% of patients cannot attain this goal with monotherapy, and by 9 years this declines to approximately 25%. The majority of patients need multiple therapies to attain these glycemic target levels in the longer term.[3]

Increasing realization of the need for optimal glycaemic control and the pitfalls of available therapeutic options in type 2 diabetes have led to active search for newer

modalities of therapy. Also the existing drugs have several limitations in terms of adverse effects.

The drug evaluated in the present study is a novel benzimidazole compound i.e. 2 nitro substituted benzimidazole. Benzimidazoles are used extensively in the treatment of human helminth infections. They have excellent safety profile. There are studies showing that benzimidazoles possess hypoglycemic activity.[4] The present study is undertaken to evaluate the anti diabetic efficacy of benzimidazoles in the treatment of experimentally induced diabetes mellitus in rats.

METHODS

Experimental Animals

Male albino wistar rats weighing 150-200 grams and of more than 3 months of age were obtained from central animal house, Osmania Medical College. The animals were housed in standard polypropylene cages with 3 animals per cage and maintained at a temperature of 24-27^{0}c with relative humidity of 30-70 % with 12 hr light dark cycle. All the animals were provided with commercially available rat normal pellet diet and water ad libitum.

The present study was approved by the Institutional Animal Ethics Committee.

Chemicals

Alloxan monohydrate (Sigma Chemical Company), 2 nitro benzimidazole (obtained in pure form from Kakatiya University) were used in the study. All other chemicals were obtained from local sources and were of analytical grade.

Acute toxicity study

Adult albino rats were used for the study. For calculation of the therapeutic dose, the dose was increased from 1 mg to 100 mg in normal rats and the therapeutic dose determined as 15 mg/kg body weight.[5] No mortality or abnormal behaviour was seen upto 100 mg. The animals were observed for a period of 14 days.

Experimental design

Grouping of animals

Animals were randomly divided into four groups, six animals in each group, which were kept separately in different cages. Individual animals were identified by a mark on the tail with a permanent marker and the cages are identified with label pasted on the cages with groupings written on them.

They are -

Group 1: normal control

- 0.5 ml Gum acacia p.o. for 7 days

Group 2: Diabetic control

- 0.5 ml Gum acacia p.o. for 7 days

Group 3: Standard group:

- 10 mg/kg bw Glibenclamide in 0.5 ml of gum acacia p.o. for 7 days

Group 4: Test group:

- 15 mg/kg 2- nitro benzimidazole in 0.5 ml of gum acacia p.o. for 7 days

Group 1 and 2 animals received only vehicle (2% Gum acacia) orally in a volume of 0.5 ml/kg and served as a normal and diabetic control respectively. Group 3 received glibenclamide (10 mg/kg, p.o.)[6] as a standard drug suspended in vehicle(gum acacia). The test compound, 2 nitro benzimidazole, suspended in vehicle(gum acacia), was administered at doses of 15mg/kg, p.o. all the drugs were administered for seven days.The animals are allowed free access to food and water but were withheld food and only given water 12 hours prior to estimation of fasting blood glucose.

Induction of diabetes

Leaving six animals which serve as normal control, experimental diabetes was induced in rest of the rats by injecting 120 mg/kg of alloxan monohydrate intraperitoneally in 0.9% w/v NaCl to over-night fasted rats. The rats were then kept for the next 24 h on 10% glucose solution to prevent hypoglycemia.[7] After 72 hours, the blood glucose levels of all rats were determined by using glucometer. The rats with blood glucose levels of 200 mg/dl were considered as diabetic and were employed for further study.

Method of blood collection

Blood is collected from the tail vein. The animal is placed in a suitable restrainer and the tail veins in the rat are made prominent by dipping the tail in water at 40-50^{0}c or rubbing the tail with xylol. A gentle aspiration or a prick with needle is made at the distal part of the tail to collect blood.[8]This method yields smaller blood volumes and is also suitable for repeated blood sampling.[9]

Estimation of blood glucose levels

Blood samples are collected for the estimation of blood sugars in rats as fasting (12 hr overnight fasting), 2hr, 4hr and 6hr after administration of the drug on day 1 and 7.

Blood glucose levels were estimated using ONE TOUCH Ultra™ Glucometer.

Statistical analysis

The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA, followed by Dunnet's test. P <0.05 was considered significant.

RESULTS

Acute Toxicity Studies

The drug 2 nitro benzimidazole was given to normal healthy rats which showed no signs of toxicity. Neurological, anatomical profile was found to be normal. They showed no significant behavioural changes. The animals were observed for a period of 72 hours. No mortality was observed.

The doses were gradually increased and maximum effect is seen at 15 mg /kg body weight per oral route. With further increase in dose, there is no significant improvement in hypoglycaemic effect.

Effects of 2 nitro benzimidazole on alloxan-induced diabetic rats for 24 hour period

With single dose administration of the test drug (group 4), the animals showed decrease in blood glucose and the percentage reduction was 67 % at 2 hr, 57% at 4 hr, 52% at 6 hr.

With single dose administration of the standard drug, the percentage reduction in blood glucose values in standard group (group 3) was 60% at 2hrs, 57% at 4 hrs, 43% at 6 hrs and 45% at 24 hrs. During this 24 hour period the animal showed no untoward effect

The p value is significant for both the groups (<0.0001). This shows that the antidiabetic activity of the test compound is higher than the standard at 2 hr and comparable at 4 hr.

The percentage decrease in blood sugar levels in group 3 administered the standard drug were 39% at 0 hr, 66% at 2 hr, 54% at 4 hr, and 37 % at 6 hr.

The percentage decrease in blood sugar levels in group 3 administered the standard drug were 26% at 0 hr, 63% at 2 hr, 49% at 4 hr, and 27% at 6 hr. The p value is significant for both the groups.

This shows that the hypoglycemic activity of 2 nitro benzimidazole is comparable with glibenclamide 2 and 4 hours after administration of the drug.

DISCUSSION

The present study was conducted to evaluate the anti-diabetic effect of 2 nitro benzimidazole. Glibenclamide was used as a standard drug and the results were compared in reference to it. The drugs were given for 7 days. The decreases in the blood sugar level were recorded as fasting, 2 hr, 4 hr and 6 hrs after administration of the drug on day 0 and day 7.

In the present study, alloxan was used as a diabetogen. It induces diabetes by destroying b-cells of the pancreas partially, through production of reactive oxygen species.[10]

Over production of glucose and decreased utilization by the tissues form the fundamental basis of hyperglycemia in diabetes mellitus.[11] This also leads to the development of microvascular and macrovacular complications.

In group 1 which is non diabetic control group no increase or decrease in blood glucose level was observed as shown in table 1 and 2. Average increase in blood glucose levels was observed in group 2 rats which are diabetic control group as shown in table 1 and 2

Table 1: Comparison of antidiabetic effect of 2 nitro benzimidazole and glibenclamide on mean blood glucose levels in diabetic rats for 24 hours.

Groups	Treatment	Mean blood glucose expressed as mg/dl			
		0 hr	2 hr	4 hr	6 hr
I.	Normal Control (2% gum acacia)	85.67± 6.76	81.56±4.89	88.67±3.95	90.67±4.22
II.	Diabetic control (Alloxan 125 mg/kg IP+2% gum acacia)	273.80±25.93	281.56±23.19	293.67±18.43	286.42±25.93
III.	Standard Group (Alloxan 125 mg/kg IP+ Glibenclamide 10 mg/kg)	161.20±13.81	111.73±14.46	127.83±16.54	137.64±18.54
IV.	Test Group (Alloxan 125 mg/kg IP+ 2-nitro Benzimidazole 15 mg/kg)	196.34±24.12	94.33±8.20	126.50±15.07	164.34±17.43
	P value		<0.0001	<0.0001	<0.0001

All values are expressed as Mean ± SEM
Groups 1, 3 and 4 are compared with group 2. Results analyzed with one way ANOVA

Table 2: Comparison of antidiabetic effect of 2 nitro benzimidazole and glibenclamide on mean blood glucose levels in diabetic rats on day 7.

Groups	Treatment	Mean blood glucose expressed as mg/dl			
		0 hr	2hr	4hr	6 hr
I.	Normal Control (2% gum acacia)	71.03±5.19	82.12±8.71	71.12±5.58	90.54±8.51
II.	Diabetic control (Alloxan 125 mg/kg IP+2% gum acacia)	270.54±20.12	297.28±28.74	277.65±22.29	282.43±28.42
III.	Standard Group (Alloxan 125 mg/kg IP+ Glibenclamide 10 mg/kg)	163.54±19.34	101.31±29.62	129.86±29.04	178.67±18.52
IV.	Test Group (Alloxan 125 mg/kg IP+ 2-nitro Benzimidazole 15 mg/kg)	199.49±16.63	111.38±26.56	141.87±19.06	205.20±26.01
	P value	<0.001	<0.001	<0.001	<0.001

All values are expressed as Mean ± SEM for 4 groups of 6 animals each.
Groups 1, 3 and 4 are compared with group 2. Results analyzed with one way ANOVA

The effect of 2 nitro benzimidazole was comparable to that of glibenclamide within 2-4 hours of administration of drugs in the diabetic rats. It produced significant fall in blood glucose levels. The maximum percentage of reduction was found at 2 hr at 66% indicating its peak action.The fasting and 6th hr values of test compound were low in comparison with glibenclamide indicating that it is not a long acting drug.

The blood glucose levels on day seven of administration of drugs show the same effects as on day 1 with comparable activity against glibenclamide at 2 and 4 hours. The fasting levels of glibenclamide were superior to the test drug.

The onset of action of test drug is immediate with peak action at 2 hours. The duration of action appears to be 4 hours. This activity profile is similar to glibenclamide.

Administering the drug for seven consecutive days did not produce any further fall in the blood sugar levels indicating that the compound doesn't have any potentiating effect on long term administration.

The probable mechanism of actions of 2 nitrobenzimidazole might be through activation of AMP-activated protein kinase (AMPK) which results in decreased gluconeogenesis and lipogenesis, increase in glucose uptake and fatty acid oxidation in skeletal muscle, inhibits hepatic glucose production in the liver.[12]

It has been seen that there is an association between oxidant activation of poly (ADP ribose) polymerase (PARP) and upregulation of known mediators of glycaemic injury. Inhibitors of PARP may have potential therapeutic roles in the prevention of diabetic complications.The benzimidazole compound is said to possess PARP activity.[13]

The other probable mechanism of action might be through inhibition of Dipeptidyl-peptidase-4 (DPP-4).[14]

Chronic studies and chronic toxicity studies have to be done for further evaluation of the drug.

CONCLUSION

The results indicate that the test compound 2 nitro benzimidazole has significant and sustained oral hypoglycaemic activity, comparable with glibenclamide in alloxan induced diabetic rats. The test drug seems like a better short acting drug and needs to be further evaluated.

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