Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20184850

Original Research Article

Effect of nicorandil, on blood glucose level in alloxan induced diabetic rats and its pharmacodynamic interaction with glibenclamide

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Received: 22 September 2018
Accepted: 22 October 2018

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ABSTRACT

Background: Diabetes increases the risk of macrovascular complications and is often associated with angina in patient. Currently nicorandil, a potassium channel opener is being frequently used for the prevention and long-term treatment of angina pectoris. Glibenclamide exerts its antidiabetic action by closing the ATP sensitive potassium channels. Simultaneous use of nicorandil may antagonizes this action and may worsens the existing diabetes. To evaluate the pharmacodynamic interaction present study has been taken to study the effect of Nicorandil, a potassium channel opener on blood glucose level of alloxan induced diabetic rats and its pharmacodynamics interaction with Glibenclamide.
Methods: Albino rats, weighing 150-200gm of male sex were used for the study. Diabetes was induced by injecting alloxan monohydrate 2% solution intra peritoneally in a dose of 150mg/kg body weight. Animal with Fasting Blood Sugar level between 250-300g/dl was selected for study and they were divided into 4 groups of 5 animals each. Group I- serving as control received 0.5ml normal saline orally for 28 days. Group II was given glibenclamide (0.5mg/kg body wt) for 28 days. Group III was treated orally with nicorandil (0.3mg/kg body wt) for 28 days. Group IV was given glibenclamide (0.5mg/kg) and nicorandil (0.3mg/kg) for 28 days. Fasting Blood Sugar level was recorded in all rats on 1st,3rd,7th,14th,21st and 28th day of the treatments.
Results: results showed that glibenclamide significantly reduce blood sugar level ($p < 0.05$) Wherase nicorandil showed rise in blood glucose level ($p < 0.05$) While the combination (glibenclamide + nicorandil) showed rise in blood glucose ($p < 0.05$) overall.
Conclusions: Nicorandil worsen the existing diabetes and to be avoided or replaced with alternative drug in case of diabetes being treated with sulfonyl urease group of drugs.

Keywords: Alloxan, Glibenclamide, Nicorandil, Sulfonylureas

INTRODUCTION

According to International diabetes federation atlas update 2015, 415 million people in the world suffer from diabetes and by 2040 it will rise to 642 million.[1] In 2000, India with 31.7 million diabetic patients topped the world with highest number of diabetic mellitus followed by China (20.8million) with the United States (17.7million) in 2nd and 3rd position respectively.[2] It is predicted that by 2030 in India diabetes status will flare up by 79.4 million.[3] Diabetes is a chronic metabolic disorder characterized by hyperglycemia, hyperlipidemia, glycosuria, and negative nitrogen balance. It is associated with significant morbidity due to specific diabetes related microvascular (retinopathy, nephropathy, neuropathy) and macrovascular complications (ischemic heart disease, stroke, peripheral vascular disease), and thus diminished quality of life. All Together they accounted for 38 million death in 2012 worldwide, 52% of mortality in South Asia, and have been projected to account for 72% of mortality in this region by 2030. Asian Indians have highest rate of coronary artery disease and often has malignant course.[4] Magnitude of the problem led to launching of National Program for Control of Cancer, Diabetes, Cardiovascular

Disease and Stroke (NPCDCS)5 by Government of India in 100 districts in 2010, with strong screening and monitoring components, and it was subsequently strengthened in 2013-2014.[5] Treatment of diabetes and cardiac illness in our population requires careful selection of drugs taking a note on possible drug interactions which can negate others action. The most common oral anti-diabetic drug used in the treatment of type 2 diabetes is sulfonylureas. Other oral hypoglycemic agent is insulin sensitizer thiazolidinediones (like pioglitazone, rosiglitazone) biguanides (like metformin) and α glucosidase inhibitors (acarbose, miglitol). Also, there are some recently developed drugs like amylin agonist (pramlintide), analog of glucagon like peptide-1 (GLP-1 analog e.g. exenatide and liraglutide), and inhibitor of Dipeptidyl peptidase-4 (DPP-4 inhibitor e.g. Sitagliptin). Cardiovascular disorder like angina pectoris, hypertension is often treated with potassium channel opener like nicorandil. ATP-sensitive potassium (KATP) channels are found in a wide variety of tissues, including skeletal and smooth muscle cells, secretory cells (such as insulin-secreting pancreatic ß-cells), cardiac myocytes and neurons. Potassium channel opener drug such as minoxidil, diazoxide, pinacidil, nicorandil and cromakalim are a chemically heterogeneous group of compounds that relaxes smooth muscle as a result of membrane hyperpolarization.[6] They act primarily on smooth muscle and mediate vasodilation and fall of blood pressure. Nicorandil increases cyclic GMP by stimulating guanylyl cyclase in epicardial coronary arteries producing vasodilatation.

KATP channel present in the pancreatic β cells is an octamer composed of four subunits of sulphonylurea receptor (SUR1) and four subunits of inwardly rectifying potassium channel (KIR6.2). KIR6.2 is the pore forming subunit of KATP channel and is active only in the presence of a regulatory subunit SUR1. This channel is rendered inactive even if one of the KIR6.2 subunits is not coupled to SUR1 subunit.[7] The reason quoted is requirement of SUR1 for the normal trafficking and surface expression of KIR6.2. SUR1 is also known to modify the affinity of KIR6.2 towards ATP.[8] It was presumed that potassium channel openers may cause hyperpolarization of pancreatic beta cells, reduce entry of Ca^{2+} through the voltage operated Ca^{2+} channel, inhibit insulin secretion by exocytosis and produce hyperglycemia. There is paucity of literature in pre-clinical studies regarding the consequences of pharmacodynamics drug interaction between glibenclamide and nicorandil on blood glucose level. The effect of potassium channel opener on blood glucose level in non-diabetic and diabetic person is also not clear. Hence, present work was undertaken to study the effect of nicorandil on blood glucose level in rat made diabetic by alloxan monohydrate and its pharmacodynamics interaction with glibenclamide.

The aim of the present endeavour was to study the effect of nicorandil on blood glucose level in alloxan induced diabetic rats and to study the pharmacodynamic interaction of glibenclamide and nicorandil on blood glucose level of alloxan induced diabetic rats.

METHODS

The present study was conducted in the department of pharmacology, MGM Medical College, Jamshedpur. Ethical clearance was obtained from Institutional Ethics Committee and Institutional Animal Ethics Committee of MGM Medical College, Jamshedpur. The interventions and investigation to be carried out in albino rats was done by adopting the terms prescribed by CPCSEA guidelines. Healthy male wistar rats weighing between 150-200grams were taken for the present study. The animals were kept in clean and dry cages, with 12 h: 12 h light-dark cycle at room temperature and humidity. They were acclimatized to the available housing condition and were fed with standard laboratory diet consisting of soaked black gram (Kala Chana) and water was given ad libitum. The cages were floored with a layer of saw dust for absorption of urine of rats. This was done because after induction of diabetes by alloxan there would be excess of urination of rats.

Inclusion criteria

- Normal rats: Adult male albino rats weighing 150-200g
- Diabetic rats: Alloxan induced diabetic male rats with blood glucose more than 250mg/dL.

Exclusion criteria

The albino rats which do not fall in the above-mentioned weight are excluded from study.

Animals were divided into four groups with five rats in each group.

Total of 25 healthy male albino rats weighing 150-200g were included in the study. The methods employed in this study to induce diabetes was chemical method using alloxan monohydrate 2%, solution which was dissolved in 0.9% of sodium chloride (normal saline) as a diluent given intraperitoneally to rats and blood glucose estimation made by using glucometer. Fasting blood glucose levels was measured before starting the treatment and after alloxan administration at 24hr, 48hr and 72hr by using glucometer. Rats with blood glucose level between 250-300 were included in the study. 3 rats did not develop hyperglycemia and 2 died two days after the procedure. The remaining 20 diabetic rats were divided into four groups of 5 rats each.

Group allocation

- Group-I: Control, alloxan induced diabetic rat was fed with 0.5ml of normal saline (oral).

- Group-II: alloxan induced diabetic rats orally fed with o.5% of normal saline +glibenclamide 0.5mg/kg body wt.
- Group-III: Alloxan induced diabetic rat orally fed with 0.5ml of normal saline +nicorandil 0.3mg /kg body wt.
- Group-IV: Alloxan induced diabetic rats orally fed with 0.5ml normal saline + glibenclamide 0.5mg /kg body wt +nicorandil 0.3mg/kg body wt.

FBS was recorded in all rats on 1st,3rd,7th,14th,21st and 28th day.

- Group-I and Group-II was compared to observe the effect of glibenclamide in diabetic rats.
- Group-I and Group-III was compared to observe the effect of nicorandil on diabetic rats.
- Group-II and Group-IV was compared to observe the effect of glibenclamide and nicorandil interaction in diabetic rats.

Method of blood collection

Blood samples were collected from the tail of rat, since it is the most venous part of body of rat. The tail of rat was cleaned with spirit cotton and then with the help of sterilized blade, it was cut 0.5mm just enough to allow one drop of blood to ooze out and was collected directly on the strip placed in the glucometer (Dr. Morepen Gluco One BG-03 Gluco meter).

Statistical analysis

The results were analysed by calculating the mean values, the standard deviation, the standard error, the 't'- test, 'p'-value and the analysis of variance (ANOVA). P Value is the standard table value of 't' at (12 - 2) = 10 degree of freedom for 0.05 (5%) level of significance, P <0.05 = significant P >0.05 = Not significant.

RESULTS

Blood glucose comparison within each group

In diabetic control group the blood glucose level was in range from 278.80±3.1mg/dl to 285.80±6.8mg/dl during the study period of 28 days. There was no significant difference in the mean values of blood glucose level from Day 0 to Day 28 in the diabetic control group (p <0.05).

In glibenclamide group (standard) the blood glucose level significantly decreased (p <0.05) with the treatment days. At Day 0 the level of glucose was 279.40±9.32mg/dl and on Day 28 it was 132.80±12.93mg/dl, which was nearly half of the control. It indicates that the rats responded with glibenclamide treatment.

Table 1: Blood glucose levels of control and treatment group during the experiment period.

Groups	Control (mg/dl)	Glibenclamide (mg/dl)	Nicorandil (mg/dl)	Glibenclamide+ Nicorandil (mg/dl)
Day 0	285.20±8.32	279.40±9.32	273.80±10.82	288.00±13.57
Day 1	282.80±9.01	247.20±29.14	280.40±10.33	282.20±13.25
Day 3	285.80±6.8	230.40±38.30	287.80±11.58	278.00±15.10
Day 7	284.20±6.7	204.80±29.38	300.40±10.62	273.20±15.12
Day 14	280.80±4.2	180.40±16.64	317.80±11.96	263.40±15.72
Day 21	278.80±3.1	149.40±18.27	331.80±11.23	252.60±17.78
Day 28	280.60±3.2	132.80±12.93	350.60±13.31	241.40±18.46
F factor between days	0.863	24.314	30.821	5.741
Significance		0.000	0.000	0.001

Value (Mean±SEM) with different superscripts within the same row differ significantly at least (p <0.05).

In Nicorandil group (test) the blood glucose level increased with the time progressed. At day 3 of treatment, no significant difference (p <0.05) was observed but after Day 5 onward a significant difference (p <0.05) was observed (273.80±10.82 mg/dl vs 350.60±13.31mg/dl) (Table 1).

When these two drugs were given in combination (glibenclamide + nicorandil), the difference in mean values of blood glucose level was found statistically significant (P<0.05) from day 14 onward. This experiment revealed that glibenclamide treatment significantly decreased the glucose level while nicorandil increased the blood glucose level. When these two drugs were used in combination a slight decrease in blood glucose was noticed. This reveals that nicorandil reduces the effect of glibenclamide.

Comparative study of blood glucose level between control and treatment groups

Blood glucose levels in glibenclamide group

When blood glucose level between each treatment day of control group and glibenclamide group was compared, it was found that the mean value of blood glucose levels was significantly lower ($p < 0.05$) on day 1 and day 3 while highly significant ($p < 0.01$) decrease was observed on Day 7 onward (Table 2).

Table 2: Blood glucose levels in glibenclamide group.

Days	Control (mg/dl)	Glibenclamide (mg/dl)	P value
0	285.20±3.72[a]	279.40±4.17[a]	0.33
1	282.80±4.03[a]	247.20±13.03[b]	0.047
3	285.80±3.23[a]	230.40±17.13[b]	0.03
7	284.20±3.00[a]	204.80±13.14[b]	0.004
14	280.80±1.88[a]	180.40±7.44[b]	0.000046
21	278.80±1.39[a]	149.40±8.17[b]	0.000098
28	280.60±1.44[a]	132.80±5.78[b]	0.000015

Value (Mean±SEM) with different superscripts within the same row differ significantly at least ($p < 0.05$).

Table 3: Blood glucose levels in nicorandil group.

Days	Control (mg/dl)	Nicorandil (mg/dl)	P value
0	285.20±3.72[a]	273.80±4.84[a]	0.09
1	282.80±4.03[a]	280.40±4.62[a]	0.7
3	285.80±3.23[a]	287.80±5.18[a]	0.75
7	284.20±3.00[a]	300.40±4.75[b]	0.023
14	280.80±1.88[a]	317.80±5.35[b]	0.001
21	278.80±1.39[a]	331.80±5.02[b]	0.0001
28	280.60±1.44[a]	350.60±5.95[b]	0.0003

Value (Mean±SEM) with different superscripts within the same row differ significantly at least ($p < 0.05$).

Table 4: Blood glucose levels in nicorandil + glibenclamide group.

Days	Control (mg/dl)	Glibenclamide+ Nicorandil (mg/dl)	P value
0	285.20±3.72[a]	288.00±6.07[a]	0.700
1	282.80±4.03[a]	282.20±5.93[a]	0.935
3	285.80±3.23[a]	278.00±6.75[a]	0.332
7	284.20±3.00[a]	273.20±6.76[a]	0.187
14	280.80±1.88[a]	263.40±7.03[a]	0.062
21	278.80±1.39[a]	252.60±7.95[b]	0.031
28	280.60±1.44[a]	241.40±8.26[b]	0.009

Value (Mean±SEM) with different superscripts within the same row differ significantly at least ($p < 0.05$).

Blood glucose levels in nicorandil group

But when we compared nicorandil group with control group significant ($p < 0.05$) rise in blood glucose level was observed from day 7 onward i.e. from 300.40±4.75 to 350.60±5.95mg/dl, whereas no significant ($p > 0.05$) rise in blood glucose level was observed on day 1 and day 3 (Table 3).

Blood glucose levels in nicorandil + glibenclamide group

When combination of drug nicorandil + glibenclamide was given, it was found that mean value of blood glucose level was reduced significantly ($p < 0.05$) on day 21 and day 28. While in day 1 to day 14 there is insignificant lowering in blood glucose level ($P > 0.05$) as shown in (Table 4).

Comparative study of blood glucose levels between treatment groups

Between glibenclamide and nicorandil groups

When comparative study of mean blood glucose was observed between the treatment groups glibenclamide and nicorandil, it was found that significant ($p < 0.05$) differences was observed from day 1 onward but highly significant after day 14 (Table 5).

Table 5: Comparative study between glibenclamide and nicorandil.

Days	Glibenclamide (mg/dl)	Nicorandil (mg/dl)	P value
0	279.40±4.17[a]	273.80±4.84[a]	0.4
1	247.20±13.03[a]	280.40±4.62[a]	0.061544
3	230.40±17.13[a]	287.80±5.18[b]	0.023788
7	204.80±13.14[a]	300.40±4.75[b]	0.001018
14	180.40±7.44[a]	317.80±5.35[b]	1.41E-06
21	149.40±8.17[a]	331.80±5.02[b]	2.77E-07
28	132.80±5.78[a]	350.60±5.95[b]	4.78E-09

Value (Mean±SEM) with different superscripts within the same row differ significantly at least ($p < 0.05$).

Table 6: Comparative study between glibenclamide and glibenclamide + nicorandil groups.

Days	Glibenclamide (mg/dl)	Glibenclamide + Nicorandil (mg/dl)	P Value
0	279.40±4.17[a]	288.00±6.07[a]	0.280825
1	247.20±13.03[a]	282.20±5.93[b]	0.050156
3	230.40±17.13[a]	278.00±6.75[b]	0.049108
7	204.80±13.14[a]	273.20±6.76[b]	0.003583
14	180.40±7.44[a]	263.40±7.03[b]	3.97E-05
21	149.40±8.17[a]	252.60±7.95[b]	1.78E-05
28	132.80±5.78[a]	241.40±8.26[b]	1.31E-05

Value (Mean±SEM) with different superscripts within the same row differ significantly at least ($p < 0.05$).

Between glibenclamide and glibenclamide + nicorandil groups

When comparative study was done between glibenclamide and (glibenclamide + nicorandil) groups it was found that significant ($p < 0.05$) differences in mean blood glucose level was observed from day 1and highly significant differences was observed from day 14 onward (Table 6).

Between Nicorandil and Glibenclamide + Nicorandil Groups

Table 7: Comparative study between nicorandil and glibenclamide + nicorandil groups.

Days	Nicorandil (mg/dl)	Glibenclamide + Nicorandil (mg/dl)	P value
0	273.80±4.84[a]	288.00±6.07[a]	0.104708203
1	280.40±4.62[a]	282.20±5.93[a]	0.816763642
3	287.80±5.18[a]	278.00±6.75[a]	0.287361844
7	300.40±4.75[a]	273.20±6.76[b]	0.013279982
14	317.80±5.35[a]	263.40±7.03[b]	0.000464963
21	331.80±5.02[a]	252.60±7.95[b]	6.57086E-05
28	350.60±5.95[a]	241.40±8.26[b]	1.34412E-05

Value (Mean±SEM) with different superscripts within the same row differ significantly at least ($p < 0.05$).

When mean blood glucose level of nicorandil and (glibenclamide + nicorandil) groups was compared, it was found that significant ($p < 0.05$) differences in mean blood glucose level was observed from day 7 onward and highly significant at day 21 and day 28 (Table 7).

Percentage of increase or decrease in blood glucose level in different treatment groups

Table 8: Percentage of increase or decrease in blood glucose levels in different treatment groups.

Days	Treatment groups		
	Glibenclamide (%)	Nicorandil (%)	Glibenclamide + Nicorandil (%)
Day 0	0	0	0
Day 1	-11.5247	2.410167	-2.01389
Day 3	-17.5376	5.112474	-3.47222
Day 7	-26.7001	9.713701	-5.13889
Day 14	-35.4331	16.06778	-8.54167
Day 21	-46.5283	21.18025	-12.2917
Day 28	-52.4696	28.04557	-16.1806

When we studied the percentage of increase or decrease in blood glucose level in different treatment groups, it was found that glibenclamide group showed a fall in blood glucose level as shown in the table. The mean fall in blood glucose level was 27.16% over 28 days which was found to be statistically significant ($p < 0.05$).

In nicorandil group, there is rapid rise in blood glucose level from 1st to 28th day which was statistically significant ($p < 0.05$) as shown in the table. The mean rise of blood glucose level was 11.8% over 28 days.

When drugs were given in combinations i.e. both glibenclamide and nicorandil there were an initial fall in blood glucose level by 2.01% and 3.4% on 1st and 3rd day, which was found to be statistically non-significant ($p > 0.05$). Later the same group showed a statistically significant ($p < 0.05$) fall in blood glucose level from 14th day to 28th day as shown in the Table 8.

DISCUSSION

Diabetes is most commonly associated with hypertension and ischemic heart disease, for which a combination of drug therapy is required. In addition to nitrates, potassium channel openers are the other group of drugs that are useful in hypertension and ischemic coronary heart disease. It has been found that hyperglycemia is one of the commonest side effects of potassium channel opener like diazoxide due to inhibition of insulin release.[9]

The mean blood glucose level of different groups was compared and it was found that in glibenclamide group the mean blood glucose level significantly decreased with the treatment days. At day 0 the level of glucose was 279.40±9.32 and on day 28 it was 132.80±12.93, which was nearly half of the control. It indicates that the rats responded with glibenclamide treatment. The same findings were also reported by Ahmed SM and Meraj et al.[9,10]

In nicorandil group the blood glucose level increased as the time progressed. Up to day 3 of treatment, no significant difference was observed but after day 5 onward a significant difference was observed (273.80±10.82 vs 350.60±13.31). Present study result is well in accordance with study done by Suresha et al.[11]

When these two drugs given in combination (glibenclamide + nicorandil) the difference in mean values of blood glucose level was found statistically significant from day 14 onward. This experiment revealed that glibenclamide treatment significantly decreased the glucose level while nicorandil increased the blood glucose level. When these two drugs were used in combination a slight decrease was noticed. This revealed that nicorandil reduces the effect of glibenclamide.

The study shows statistically significant interaction between nicorandil and glibenclamide. This finding contradicts the earlier thought that nicorandil has specificity for the KATP channels in the blood vessels and not for the KATP channels present in the pancreas. Similar finding was also reported by Ahmed SM and Kasono et al, also reported that nicorandil improved STZ-induced diabetes mellitus in vivo and protected b-cells against the toxic action of STZ in vitro. There in vivo data showed that 4 weeks of oral treatment with nicorandil to STZ-DM rats resulted in decreased blood glucose levels, retained plasma insulin concentrations, preservation of islet b-cells, and prevention of body weight loss compared with rats in the STZDM group that did not receive nicorandil treatment.[9,12]

Thus because of pharmacokinetic and pharmacodynamics variations between animal and human species further studies are required to substantiate these results in diabetic

subjects where the minimum number of subjects required in each group is greater than 20.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. IDF. Diabetes Atlas, 8th ed. Available at: http://www.idf.org/idf-diabetes-atlas-seventh-edition2015 accessed on 20/9/2016.
2. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australasian Med J. 2014;7(1):45-8.
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004 May 1;27(5):1047-53.
4. Enas EA, Mehta J. Malignant coronary artery disease in young Asian Indians: thoughts on pathogenesis, prevention, and therapy. Clinical Cardiol. 1995 Mar;18(3):131-5.
5. National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular diseases and Stroke (NPCDCS). Operational Guidelines. Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. 2011. Available at: http://health.bih.nic.in/Docs/ Guidelines/Guidelines NPCDCS.pdf.
6. Mannhold R. KATP channel openers: Structure-activity relationships and therapeutic potential. Medicinal Res Rev. 2004 Mar;24(2):213-66.
7. Zhuo ML, Huang Y, Liu DP, Liang CC. KATP channel: relation with cell metabolism and role in the cardiovascular system. Int J Biochem Cell Biol. 2005 Apr 1;37(4):751-64.
8. Sandhiya S, Dkhar SA. Potassium channels in health, disease & development of channel modulators. Indian J Med Res. 2009 Mar 1;129(3):223.
9. Ahmed SM. Study of influence of nicorandil on hypoglycemic action of glibenclamide in alloxan induced diabetic rats. Int J Basic Clin Pharmacol. 2016;5(5):2146-52.
10. Meraj T, Khatoon M, Reddy NAR. Influence of fluoxetine on pharmacokinetics and pharmacodynamics of glibenclamide in diabetic rats. Asian J Pharm Clin Res. 2014;7(2):76-9.
11. Suresha RN, Mohammed S, Jayanthi MK, Kalabharathi HL, Satish AM, Pushpa VH, et al. Effect of nicorandil on basal glucose levels and after glucose challenge in normal euglycemic albino wistar rats. Int J Pharmacy Pharmaceuti Sci. 2014;(6):723-5.
12. Kasono K, Yasu T, Kakehashi A, Kinoshita N, Tamemoto H, Namai K, et al. Nicorandil improves diabetes and rat islet beta-cell damage induced by streptozotocin in vivo and in vitro. Eur J Endocrinol. 2004 Aug 1;151(2):277-85.

Cite this article as: Soni. Effect of nicorandil, on blood glucose level in alloxan induced diabetic rats and its pharmacodynamic interaction with glibenclamide. Int J Basic Clin Pharmacol 2018;7:2378-83.