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THE EFFECT OF NIFEDIPINE ON ORAL GLUCOSE INDUCED GLYCAEMIC CHANGES IN NORMAL ALBINO RATS

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ABSTRACT

Aims: To determine effect of nifedipine on blood glucose levels through oral glucose tolerance test in normoglycemic albino Rats and magnitude of its effect on basal v/s glucose induced glycemic value. Methods: Normal adult albino rats of either sex weighing between 150 – 200g were randomly divided into 2 groups(n=6); Control(distilled water-1ml/rat) and Test(nifedipine-1.5mg/kg BW) and the respective drug given orally for 3 days. They were fasted overnight but with water before the 3rd day. On the 3rd day, 2 hours after the last drug dose , OGTT was performed with glucose 0.6g/kg BW. Results:The average CBG of the nifedipine group was higher compared to control group at 60, 120 and 150 minutes progressively with maximum hyperglycaemia (CBG 37.98%) at 60 minutes after glucose administration. Conclusion: Nifedipine worsens glycaemic control in normal rats at all hours of glucose challenge. Extending this observation in human beings with overt diabetes mellitus/ high risk diabetics/ prediabetics, it is suggested to limit the use of nifedipine in situations unless absolutely necessary since it induces hyperglycaemia even in normoglycaemic rats by a postulated mechanism of inhibition of both basal and glucose induced insulin secretion significantly.

KEY WORDS: CBG(capillary blood glucose), glycemic changes, nifedipine, oral glucose tolerance test.

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INTRODUCTION

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia due to ineffective insulin secretion or action, dyslipidemia with or without ketonemia and altered metabolism of carbohydrates, lipids and proteins [1]

The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems and is the leading cause of end stage renal disease, non traumatic lower extremity amputation , adult blindness and also predisposes to cardiovascular diseases like hypertensive heart disease, coronary artery disease, cardiomyopathy, CCF etc., With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future [2]

The International diabetes federation has predicted that the number of diabetics will increase from 240 million in 2007 to 380 million in 2025 and that the number of diabetic patients in India are going to be more than doubled from 19 million in 1995 to 40.9 million in 2007 and is projected to increase to 69.9 million by 2025. So India will be the diabetes capital of the world indicating the global population is approaching the midst of diabetes pandemic. [3]

Type II DM is at present one of the most challenging health care problems, which requires optimum management.At present the treatment of diabetes mellitus includes insulin, sulfonylureas, biguanides, α-glucosidase inhibitors, DPP-4 inhibitors, thiazolidinediones, GLP-1 receptor agonists, amylin agonists, medical nutrition therapy and lifestyle modification. [4]

The comorbid conditions associated with DM is not only dependant on tight glycemic control but also on the total duration of diabetes in the patient.[5] The therapy of comorbid conditions induces complexity in managing DM patients. eg., hypertensive heart disease by Angiotensin Converting Enzyme inhibitors/calcium channel blockers/ angiotensin receptor blockers etc, dyslipidemias by HMG CoA reductase inhibitors,fibric acid derivatives, coronary artery disease by antiplatelet drugs etc.,

The coexistence of hypertension and diabetes is frequent.[6, 7]. Cardiovascular disease accounts for 40 percent of overall mortality in the United States and is the leading cause of death among persons with type II DM. [8]

The choice of antihypertensive agents may also affect the risk of cardiovascular events. [8] calcium- channel blockers are among the most frequently prescribed antihypertensive medications since long but increasingly being used over the past decade for hypertension as are angiotensin converting enzyme inhibitors and more than β-blockers. [8]

Insulin released from resting cell is minimal and sufficient to maintain the normal blood glucose level. The rate of insulin secretion at any glucose concentration is high(glucose induced) and optimal at the end of 1 hour of glucose administration.

Ca++ ions play a vital role in biological process throughout the body by entry into the cell through L and T type of voltage gated Ca++ channels like cardiac, smooth muscle, CNS etc., [9, 11] Of them one of the important activity is to release insulin from the β cells of pancreas. [10, 12, 13]

Insulin is secreted from human pancreas by glucose entry into β cell through GLUT-2 which results in inhibition of ATP-sensitive K^+ channel resulting in depolarisation of β cells which increases Ca^{++} entry through voltage sensitive L type calcium channels into the β cells and

also releasing Ca++ from intracellular binding sites, such as the internal surface of the cell membrane, sarcoplasmic reticulum and mitochondria of the β-cell resulting in release of insulin by degranulation of stored vesicles. [14]

Insulin secretory profiles reveal a pulsatile pattern of hormone release, with small secretory bursts occurring about every 10 min, superimposed upon greater amplitude oscillations of about 80–150 min. Incretins(GLP-1) are released from neuroendocrine cells of the small intestinal

tract following food ingestion and amplify glucose-stimulated insulin secretion when the blood glucose is above the fasting level. and suppress glucagon secretion. [15]

. The calcium channel blockers block voltage dependant Ca^{++} channels and inhibit the influx of calcium ions through the cell membrane not only directly decreasing the concentration of cytoplasmic calcium but also decrease calcium release from intracellular stores. [16]

The three major class of CCBs are phenylalkylamines, dihydropyridines and benzylalkylamines the most commonly used drugs being dihydropyridines like nifedipine, amlodipine, clindapine, lacidipine etc,. They are indicated for treatment of variety of conditions like pulmonary and systemic hypertension, hypertrophic cardiomyopathy, cerebral arterial spasm, end stage renal disease in addition to various non cardiovascular conditions. [17,18]

Of them one of the short acting CCB-nifedipine is used very commonly in the treatment of cardiovascular diseases with or without diabetes. Nifedipine acts by binding to α1 subunit,of L-type voltage gated calcium channels. Binding of the drug reduces the frequency of opening in response to depolarization resulting in marked decrease in transmembrane calcium current, which in turn results in smooth muscle relaxation and in cardiac muscle with a

reduction in contractility and decreases sinus node pacemaker rate and atrioventricular node conduction velocity. The duration of action being 4-6hrs, its half life is 1.8hrs and elimination half life is 2-5hrs. [19]

Series of articles and commentaries suggest that calcium-channel blockers, including second-generation dihydropyridines, such as amlodipine and nisoldipine, may be harmful, particularly in patients with hypertension and DM. [20]

Nifedipine has been widely used in Hypertension treatment of diabetics, although a possible influence on glucose tolerance and insulin secretion (Basal & Glucose induced) is not been clearly elucidated, particularly in the experimental animals.

Based on the above facts,the controversy that remains is the effect of CCBs on basal insulin secretion, meal induced/ glucose induced insulin secretion, the present study is undertaken to determine the effect of nifedipine on glycemic levels through glucose challenge in albino rats hypothetically considering that Calcium channel blockers must impair insulin secretion.

MATERIALS AND METHODS

Chemical and drugs:

Nifedipine 1.5 mg/kg BW – orally
Glucose 0.6 gm/kg BW – orally
Distilled water

ANIMALS

Adult wistar Albino rats weighing 150-200 g were used and divided into two groups control and test, each group having 6 rats. The animals were acclimatised for 10 days before being used for the experiment. They were housed in a room with controlled temperature and a 12-hour light/ dark cycle. The animals were maintained on a standard dry pellet diet and water ad libitum. The experimental protocol was approved by the institutional animal ethics committee and was executed according to the guidelines of committee.

EXPERIMENTAL DESIGN

Rats were divided into control and test groups to study the effect of glucose induced glycemic changes in normal rats following oral administration of distilled water and nifedipine respectively. The rats were fasted overnight but provided water *ad libitum*. The control group of rats received 1 ml of tap water and the test group received nifedipine everyday in the dose of 1.5 mg/Kg BW for 3 days.On the third day, 2 hours after third dose of drug administration both the groups of rats were administered oral glucose in the dose of 0.6 gm/Kg BW. The blood glucose levels were measured at 0, 60 and 150 minutes after glucose administration (slight

modification in OGTT) by rat tail snipping method using ACCUCHEK glucometer.

OGTT:[21]

The oral glucose tolerance test is a measure of the glucose induced insulin secretion mediated glycemic control alteration. This study used OGTT for normal rats with some modifications to the standard method (Du vigneaud and Karr, 1925) to assess the effect of nifedipine on glucose induced glycemic control alteration.

Both the groups of rats were subjected to OGTT. The control group rats were given distilled water and the test rats were given nifedipine for 3 days. All rats were fasted overnight at water before the 3rd day. On third day, 2 hours after the last dose of the respective drug , OGTT was performed. After 30 minutes, all the rats were given glucose (0.6g/kg body weight) orally using gavage tube. Following this, the serum glucose of blood sample from tail vein (obtained by tail snipping) was estimated at 0, 60 and 150 minutes.

STATISTICAL ANALYSIS

The effect of the drug under study was presented by calculating the mean and S.D of the outcome parameters.one way analysis of variance (ANOVA) and independant sample T tests were applied to see the differences between any two groups at a time. Tests of significance were carried out at 5% level.

RESULTS

As shown in table 1, The mean CBG of Test group (Nifedipine) rats are significantly higher($P<0.001$) at all times of the glucose challenge i.e. 0, 60, 150 minutes from the time of administration of glucose compared to the control group. The highest worsening/hyperglycaemia is seen at 60 minutes which is 37.98% higher than the control group, followed by 0 minutes (33.44%) and 150 minutes.(4.18%)

Table 1

Table depicting CBG values of test and control group expressed as mean+/-SEM

Sl.no	Time since administration of glucose in minutes	Mean CBG(mg/dl) +/- SEM		T v/s C mg/dl	% change of CBG of T over C
		Control group(C) (n=6)	Test group(T) (n=6)		
1.	0	64.8 +/- 1.739	97.37+/- 3.646	T>C	33.44%
2.	60	83.23+/- 2.640	134.2 +/- 4.989	T>C	37.98%
3.	150	73.2+/- 3.468	76.4 +/- 2.880	T>C	4.18%

The difference in CBG values between 0 and 60 minutes in the test group is almost double that of control (36.83mg/dl in test and 18.43mg/dl in the control group). Whereas the difference in CBG values between 60 and 150 minutes in the test group is more than 5 times that of control(57.8mg/dl in test and 10.03mg/dl in control). Similarly difference between 0
and 150 min in test group is more than twice that of control(20.97mg/dl in test and 8.4mg/dl in control).(table 2)

Table 2
Table depicting difference in CBG values between various time intervals

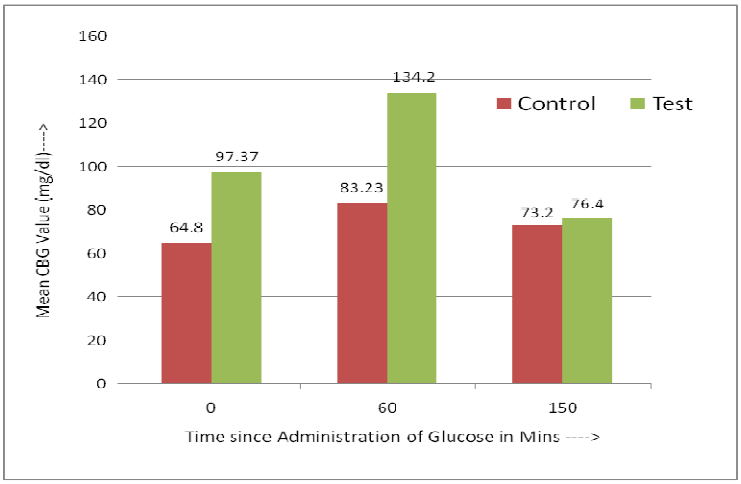
Sl.no	Time interval between	Difference in CBG values (mg/dl)	
		Control	Test
1.	0-60 min	18.43	36.83
2.	60-150min	10.03	57.8
3.	0-150 min	8.4	20.97

The difference in CBG values at all the time intervals between test & control respectively indicate Hyperglycemic action of Nifedipine except between 150 – 60 min of test & control respectively and is of very mild Hypoglycemia (hyperglycemic action of Nifedipine at 150 min of glucose administraction is compared to 60 min) (table 3)

Table 3
Table depicting difference in CBG values between various time intervals of test & control respectively

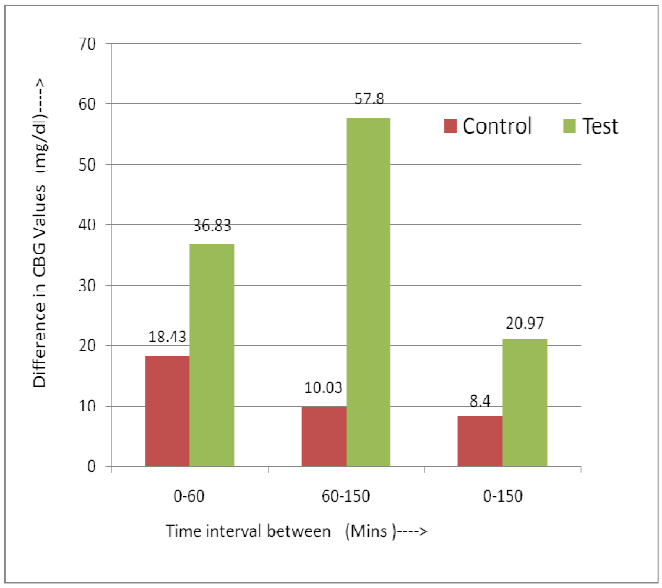
Sl.no	Time interval Between Test & Control respectively	Difference in CBG values (mg/dl)
1.	0-0 min	32.57
2.	0- 60 min	14.14
3.	0- 150 min	24.17
4	60- 0 min	69.41
5	60-60 min	50.97
6	60-150 min	61
7	150- 0 min	11.6
8	150-60 min	-6.83
9	150 -150min	3.2

Figure 1
Depicting the % change in CBG levels of test and control groups at different time intervals



Bar diagram showing the effect of nifedipine on plasma glucose concentration of normal rats in an oral glucose concentration test compared to control at 0, 60 and 150 minutes. Values are mean+/- SEM (n=6). P<0.001 compared to control group where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

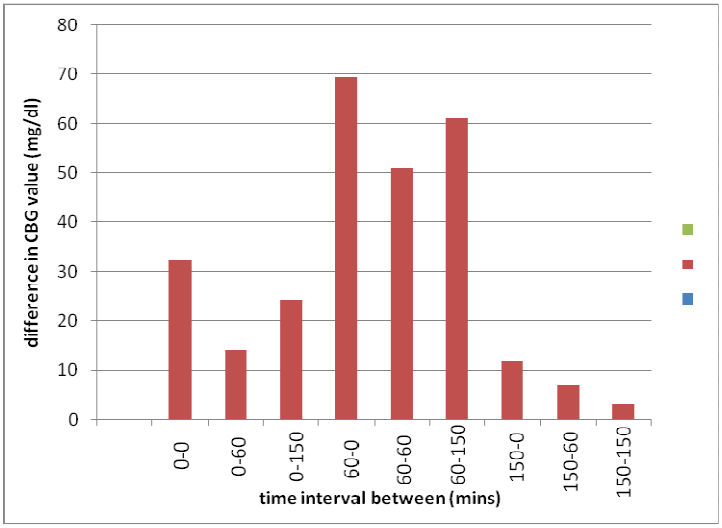
Figure 2
difference between CBG values of test and control at different time intervals



Bar Diagram showing difference in blood glucose levels between various time intervals of 0-60, 60-150 and 0-150 minutes among control and nifedipine group

Figure 3

The relationship of CBG values compared at different time intervals of Test and Control and the difference between CBG values of Test and Control.



Bar diagram showing difference in plasma glucose levels between various time intervals of test and control groups respectively.

DISCUSSION

In this study,it was observed that Nifedipine inhibits the basal insulin secretion reflected by higher CBG levels at 0 hr of glucose administration compared to control. It also affects the glucose-induced insulin secretion which is maximal at 1 hour after glucose administration reflected by high CBG levels at the end of first hour compared to control . CBG comes back to near normal level after 2 ½ hrs of oral glucose administration which corresponds to 4 to 4 ½ hrs after oral Nifedipine administration. This indicates that the inhibition of insulin secretion by Nifedipine is maximum after 3 hrs (1 hr after glucose challenge) and sustains till 4 ½ hrs (2 ½ hr after glucose challenge) of its administration So nifedipine when used in diabetics on oral hypoglycemics like sulphonylureas, gliptins, GLP analogues (secretagogues), these OHD must be given 4 ½ to 5 hrs after administration of Nifedipine.

The quantum of hyperglycaemic effect of nifedipine between 0-1 hour is almost doubled compared to control group but the quantum of hyperglycemia between 1-2 ½ hour is more than 5 times indicating maximum hyperglycaemic effect at 1 hr and sustained effect of nifedipine upto 4 ½ hrs after its administration, but the hyperglycaemic value between 0-2 ½ hrs of glucose administration is little more than twice that of control group re-establishing the hyperglycaemic effect of nifedipine even at the end of 4 ½ hrs. Gradually the quantum of hyperglycemia starts decreasing to reach just above two times the control value at the end of 2 ½ hrs after glucose administration indicating the possibility of decreased effect of nifedipine at this time.

So the implication is that as nifedipine affects basal insulin secretion also along with glucose induced Insulin secretion, the use of nifedipine is to be justified in non-diabetics, prediabetics, high risk diabetics and diabetic patients.

Because of the above demonstrated hyperglycaemic effect of nifedipine in animals, it may worsen glycaemic control in well controlled, uncontrolled diabetes mellitus, prediabetics, high risk diabetics(decreased basal & glucose indused insulin secretion – hyperglycemic values at 60 min & later) and may be even in normoglycemic

individuals (decrease basal insulin secretion – hyperglycemic values at 0 hr of OGTT). Also using this with other OHGs may need dose escalation of the OHGs to compensate glycaemic control worsening caused by nifedipine mediated inhibition of insulin secretion. This study adds a modest word of caution against use of Calcium channel blockers especially nifedipine in diabetes mellitus unless absolutely necessary.

CONCLUSION

Nifedipine worsens glycaemic control in normal rats at all hours of glucose challenge affecting both Basal & Induced Insulin secretion. So the implication follows that in human beings with well controlled Diabetes, uncontrolled diabetes mellitus, prediabetics, high risk diabetics, and may be even in normoglycemic individuals use of Nifedipine is avoided unless absolutely necessary, since it induces hyperglycaemia even in normoglycaemic rats by a postulated mechanism of inhibition of both basal and glucose induced insulin secretion significantly.

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