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EFFECT OF AMLODIPINE ON ORAL GLUCOSE INDUCED GLYCEMIC CHANGES IN NORMAL ALBINO RATS

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ABSTRACT

Objective: To determine the effect of amlodipine on blood glucose levels through oral glucose tolerance test in normoglycemic albino Rats and the magnitude of its effect on basal v/s glucose induced glycemic value compared to control.

Methods: Rats were divided into control and test groups to study the effect of glucose induced glycemic changes in normal rats following oral administration of amlodipine. The control group received 1 ml of distilled water everyday, test group received amlodipine everyday in the dose of 1.5 mg/Kg BW for 3 days.On the third day, 2 hours after drug administration both groups 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 by rat tail snipping method using ACCUCHEK glucometer.

Results: The mean CBG of Test group is significantly higher(P<0.001) at all times of the glucose challenge i.e. 0, 60, 150 minutes from the time of glucose administration compared to control group. The optimal hyperglycemia was seen at 60 minutes which is 32.76% higher than the control group, followed by 0 minutes (29.41%) and 150 minutes (7.92%).

Conclusion: Amlodipine worsens glycaemic control in normal rats at all hours of glucose challenge. Extending this to human beings, whether with impaired glucose tolerance or overt diabetes mellitus, it is suggested to limit the use of amlodipine to 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.

INTRODUCTION: Diabetes mellitus (DM) consists of group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease [1]. The pathophysiology of the disease is complex, with association at various degrees of an insulin-resistance state, defect in insulin-secretion and loss of β cell mass and perhaps modification in postprandial hyperglycaemic kinetics [2].

In spite of the introduction and extensive utilization of hypoglycaemic agents, diabetes and the related complications continue to be major health problem worldwide [3]. According to International Diabetic Federation the estimated diabetes prevalence for 2010 has risen to 285 million, representing 6.4% of the world's adult population, with a prediction that by 2030, the number of people with diabetes will have risen to 438 million.

India has been declared as the "Diabetic capital of world". Currently 40.9 million people in India suffering from diabetes and by 2030 there would be 79.44 million diabetics in India alone. It is estimated that by the year 2030, diabetes is likely to be the seventh leading cause of death accounting 3.3% of total deaths in the world [4].

Both type 1 and type 2 diabetes are known to be multifactorial diseases caused by a combination of genetic (inheritance) and environmental (diet and lifestyle) factors. In fact, chronic hyperglycemia has been established to be the principal cause of diabetic microvascular and macrovascular complications along with the total duration of diabetes [5].

Type II DM is at present one of the most challenging health care problems, which requires optimum management.At present treatment of diabetes mellitus includes insulin, sulfonylureas, biguanides, α-glucosidase inhibitors, DPP-4 inhibitors, thiazolidine-diones, GLP-1 receptor agonists, amylin agonists, medical nutrition therapy and lifestyle modification [6]

Hypertension is a common condition among diabetic patients. Hypertensive diabetics are about twice as likely to experience cardiovascular events as nondiabetic counterparts. 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 [7]. Treatment of hypertension is strongly recommended in subjects with type 2 diabetes, in whom blood pressure goals are set at levels lower than nondiabetic individuals. As a consequence, the choice of antihypertensive agent is a daily task for diabetologists and practitioners [8].

Such a choice should carefully take into account the expected benefits and potential adverse effects of available medications. In this respect, much emphasis has been given in recent years to the undesirable metabolic effects of some largely used antihypertensive agents like β-blockers or thiazide diuretics associated with a deterioration of glucose tolerance and a more atherogenic serum lipoprotein profile. Among available antihypertensive drugs, angiotensin converting enzyme inhibitors seem to be devoid of unfavorable effects on glucose and lipid metabolism. As for calcium channel blockers, their effects on glucose/lipid metabolism in type 2 diabetes are poorly understood [8]. Calcium-channel–blockers are indicated for the treatment of a variety of cardiovascular diseases, including angina pectoris, systemic and pulmonary hypertension, certain cardiac arrhythmias, and Raynaud's phenomenon. At present, CCBs are among the most frequently prescribed antihypertensive medications in the country [9].

However, although CCBs are an important part of the therapeutic armamentarium against cardiovascular diseases, concern has been aroused about these drugs, particularly short-acting dihydropyridine derivatives.

Recent studies have focused attention on the possibility that some of these agents may increase the risk of cardiovascular events in patients without diabetes. Despite their potential benefits, much controversy has arisen recently regarding calcium-channel blockers [7].

In the Fosinopril Amlodipine Cardiovascular Events Trial (FACET), the relative benefits of fosinopril and amlodipine were compared in 380 hypertensives with non-insulin dependent diabetes. The patients receiving fosinopril had a significantly lower risk of major cardiovascular events than those receiving amlodipine [10].

In 1995, a meta-analysis suggested that short-acting dihydropyridines may provoke rather than prevent myocardial infarction in patients with coronary heart disease. This study sparked a controversy, which has been fueled by a series of articles and commentaries suggesting that CCBs, including second-generation dihydropyridines, such as amlodipine and nisoldipine, may be harmful, particularly in patients with hypertension and diabetes mellitus [11].

Calcium channel blockers are a class of drugs that disrupt the conduction of calcium ($Ca2+$) channels [12]. They act by blocking voltage-gated calcium channels (VGCCs) in cardiac muscle and blood vessels [12]. Different types of calcium channels play an important role in the various cellular activities including release of insulin from β cells of pancreas [12, 13, 14]. This adds to the pharmacodynamics of insulin, that the same calcium channels which are responsible for release of insulin may be blocked by using CCBs [12].

Insulin released from resting cell is minimal. The rate of insulin secretion at any glucose concentration is high. Insulin is secreted from the human pancreas by glucose entry into the β cell through GLUT-2 which results in inhibition of ATP-sensitive K^+ channel resulting in depolarisation of β cells. This increases Ca^{++} entry resulting in release of insulin by degranulation [15].

Because calcium plays an essential role in hormone metabolism and especially in carbohydrate homeostasis and glucose- induced insulin secretion, calcium channel blocking agents might also interfere with metabolic control and are likely to impair the release of insulin- basal, stimulated or challenge with glucose or by oral hypoglycaemic drugs. Data from in vitro studies and humans with insulinomas suggest that calcium antagonists may increase serum glucose levels [16]. It is therefore important to look for any relation between calcium channel blockers and occurrence of hyperglycaemia. As amlodipine is the most commonly prescribed calcium channel blocker this study was planned to monitor the effect of amlodipine on blood sugar levels of albino rats [12].

Amlodipine belongs to class of dihydropyridines of calcium channel blockers. It is a long-acting drug used as an anti-hypertensive and antianginal drug both in diabetics and nondiabetics, acts by relaxing smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure [12].

Calcium antagonists have been widely used in antihypertensive treatment of diabetics, although a possible influence on glucose tolerance and insulin secretion is unknown and the effect of CCBs on glucose tolerance and insulin sensitivity has not been clearly elucidated, particularly in the experimental animals.

Therefore, the effect of the calcium antagonist amlodipine on glucose tolerance and insulin secretion (75 g oral glucose tolerance test) was evaluated in albino rats.

We hypothesized that amlodipine administration may impair glucose tolerance and insulin levels after glucose challenge in normal albino rats.

The oral glucose tolerance test (OGTT) is an established method to test the integrity of β-cell function to release insulin. A glucose tolerance test involves measurement of blood glucose concentration 2 hrs after a load of 75g of glucose has been taken orally in the morning after an overnight fast lasting 10 to 16h [17].

MATERIALS AND METHODS:

Chemical and Drugs:

Amlodipine 0.16 mg/kg BW – Given orally
Glucose 0.6 gm/kg BW – Given orally
Distilled water

Animals: Adult wistar Albino rats weighing 150-200 g were used and divided into two groups control and test, each group containing 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/12 hour 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 for the purpose of control and supervision on experiments in animals.

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 amlodipine respectively. The rats were fasted overnight but provided water *ad libitum*. The control group of rats received 1 ml of distilled water every day and the test group of rats received amlodipine everyday in the dose of 0.3mg/rat 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 with slight modification in OGTT by rat tail snipping method using ACCUCHEK glucometer.

Statistical Analysis: The effect of the drug under study was presented by calculating mean and S.D of the outcome parameters. One way analysis of variance (ANOVA) and independent 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. SPSS for windows (version 16) was applied in the statistical analysis.

RESULTS:

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+/- SD		T v/s C	% change of CBG of T over C
		Control group(C) (n=6)	Test group(T) (n=6)		
1.	0	64.8+/- 1.739	91.8+/- 6.089	T>C	29.41
2.	60	83.10 +/- 2.687	123.6+/- 5.205	T>C	32.76
3.	150	73.2+/-3.468	79.5 +/-3.460	T>C	7.92

*P<0.001. The mean CBG of Test group (Amlodipine) 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 32.76% higher than the control group, followed by 0 minutes (29.41%) and 150 minutes (7.92%).

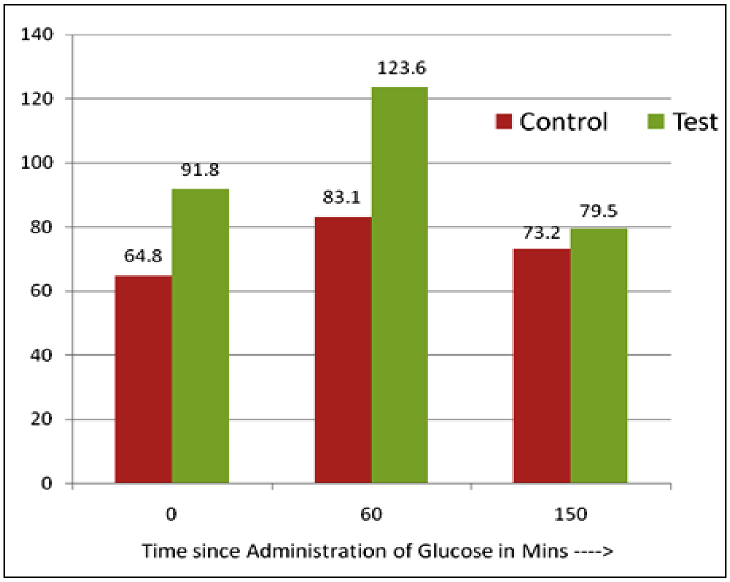

FIGURE 1: DEPICTING THE % CHANGE IN CBG LEVELS OF TEST AND CONTROL GROUPS AT DIFFERENT TIME INTERVALS

Bar diagram showing the effect of amlodipine 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.

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	18.3	31.8
2.	60-150min	9.9	44.1
3.	0-150 min	8.4	12.3

The difference in CBG values between 0 and 60 minutes in the test group is almost double that of control (31.8mg/dl in test and 18.3mg/dl in the control group). Whereas the difference in CBG values between 60 and 150 minutes in the test group is more than 4 times that of control (44.1 mg/dl in test and 9.9mg/dl in control). Similarly difference between 0 and 150 min in test group is more than the control (12.3mg/dl in test and 8.4mg/dl in control).

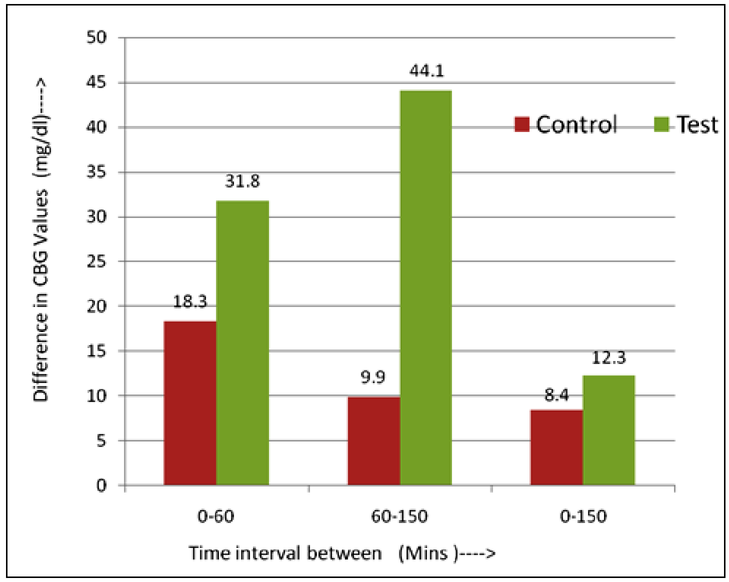

FIGURE 2: DIFFERENCE BETWEEN CBG VALUES OF TEST AND CONTROL AT DIFFERENT TIME INTERVALS

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

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	27
2.	0- 60 min	8.7
3.	0- 150 min	18.6
4	60- 0 min	58.8
5	60-60 min	40.5
6	60-150 min	50.4
7	150- 0 min	14.7
8	150-60 min	-3.6
9	150 -150min	6.3

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

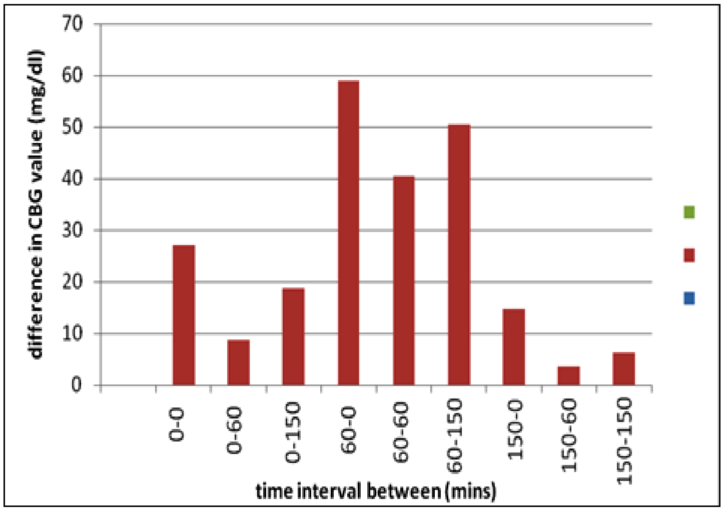

FIGURE 3: THE RELATIONSHIP OF CBG VALUES COMPARED AT DIFFERENT TIME INTERVALS OF TEST AND CONTROL AND THE DIFFERENCE BETWEEN CBG VALUES OF TEST AND CONTROL

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

DISCUSSION: In this study, it is observed that amlodipine inhibits the basal insulin secretion reflected by higher CBG levels at 0 hr of glucose administration. 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. 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 amlodipine administration. This indicates that the inhibition of insulin secretion by Amlodipine is maximum after 3 hrs and sustains till 4 to 4 ½ hrs of its administration.

The quantum of hyperglycaemic effect of amlodipine between 0-1 hour is almost doubled compared to control group but the quantum of hyperglycemia between 1-2 ½ hour is more than 4 times indicating maximum hyperglycaemic effect at 1 hr and sustained effect of amlodipine upto 4 ½ hrs after its administration but the hyperglycaemic value between 0-2 ½ hrs of glucose administration is little more than that of control group re-establishing the hyperglycaemic effect of amlodipine even at the end of 4 ½ hrs.

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

Because of the above demonstrated hyperglycaemic effect of amlodipine, it may be advisable to minimize the use of this in diabetes mellitus as it may worsen glycaemic control in well controlled as well as uncontrolled diabetes mellitus. Also using this with other OHGs may need dose escalation of the OHGs to compensate glycaemic control worsening caused by amlodipine mediated inhibition of insulin secretion after a thorough clinical trial in human population. This study adds a modest word of caution against use of Calcium channel blockers especially amlodipine in diabetes mellitus unless absolutely necessary.

CONCLUSION: Amlodipine worsens glycaemic control in normal rats at all hours of glucose challenge affecting both Basal & Induced Insulin secretion. Extending this to human beings, whether with impaired glucose tolerance or overt diabetes mellitus, it may be suggested to limit the use of amlodipine to situations where it is 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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