

Effect of flunarizine on blood glucose levels in normal albino rats through oral glucose tolerance test

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ABSTRACT

Background: Flunarizine is commonly used for migraine prophylaxis. It is a calcium channel blocker which blocks the L-type, T-type and N-type of calcium channels of pancreatic beta cells and other cells. It is believed to cause alterations in blood glucose levels secondary to its effect on calcium channel dependent insulin secretion.

Objective: To evaluate the effect of flunarizine on blood glucose levels in normal albino rats through oral glucose tolerance test (OGTT)

Materials and Methods: Flunarizine and distilled water were given orally for 5 days to the test (T) and control (C) groups of 6 normal albino rats respectively (N = 6). OGTT was conducted on both the groups on the 5th day and blood glucose levels were analyzed at 0, 60 and 150 minutes. Data was analyzed with one way ANOVA and independent samples t-test.

Results: Flunarizine caused hyperglycemia at all durations of the OGTT with a maximum difference of 26% at 0 hour. The extent of worsening was maximum at 60 - 0 minute interval in both the instances i.e. test group value compared with control (T-C) and control values compared with itself (C-C). The comparison of extent of hyperglycemia revealed that T-C showed 17% (47mg/dl) more hyperglycemia than the C-C (40 mg/dl).

Conclusion: Flunarizine has hyperglycemic effects in normal albino rats when given for 5 consecutive days orally even at the dose used for prophylaxis of migraine in human beings. A word of caution is thus advised when using flunarizine in impaired glucose tolerance or diabetic subjects.

Key words: Flunarizine, hyperglycemic, blood glucose level, oral glucose tolerance test.

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INTRODUCTION

Diabetes mellitus is widely prevalent with 90% of the cases worldwide being type 2.^[1] Drug induced diabetes mellitus is fairly common.^[2] Some calcium channel blockers are known to cause hyperglycemia.^[3]

Migraine a disorder with complex pathophysiology involving neuronal and vascular mechanisms has been found to be associated with diabetes.^[4] Some studies report a lower prevalence^[4,5] and others higher prevalence of migraine in diabetes.^[6] Insulin resistance, a causal factor for diabetes, also exists in non obese migraine patients.^[6]

Flunarizine is one of the most commonly used drugs in India for the prophylaxis of migraine^[7] with calcium channel blocking, antihistaminic, antiarrhythmic and antiserotonin effects. Its peak plasma levels appear 2 - 4 h after oral administration, when given chronically it has been found to have an elimination half life of around 19 days. Drowsiness and weight gain are its two most common side effects.^[8] It is used for variable periods of 4 to 10 months.^[9]

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The effect of flunarizine is evidenced by the reduction in the transmembrane fluxes of calcium in situations where calcium is stimulated to enter the cell in excess. Flunarizine increases cerebral blood flow and reduces the hypoxic effects on brain. However, the central nervous system concentration attained by flunarizine is less compared to other organs like pancreas and liver where significant concentrations are attained.^[10] Thus, such significant increase in cerebral blood flow as caused by flunarizine may not completely be explained by a direct effect on cerebral vasculature alone. Flunarizine blocks T-type, L-type and N-type calcium channels in various tissues including pancreatic beta cells.^[11-13] Its selectivity in calcium channel blockade is N-type, T-type and L-type in the ascending order (i.e. $N < T < L$).^[14] It is also a potent calmodulin inhibitor.^[15]

Pancreatic β -cells express L-type, T-type and N-type calcium channels. L-type are mainly involved in glucose induced insulin secretion and its reduction decreases insulin secretion.^[16] T-type channels influence the calcium influx into pancreatic β - cells thus influencing glucose stimulated insulin secretion and affects basal intracellular calcium levels thus affecting basal insulin secretion.^[17] Insulin secretion alteration is evidenced by glucose challenge induced blood glucose level changes. Calmodulin is involved in stimulus-secretion coupling for glucose - induced insulin release.^[10,18]

Literature search produced conflicting data regarding the association of calcium channel antagonists and diabetes or glucose intolerance^[3,19] which may differ with individual drug.^[3] It may depend on the type of calcium channel blocked, the selectivity and the concentration attained by the drug in the pancreas. Flunarizine's effect on carbohydrate metabolism is not completely researched. Hence, this study was conducted to evaluate

flunarizine's effect on blood glucose levels in normal rats through oral glucose tolerance test.

MATERIALS AND METHODS

Experimental animals

Adult albino rats of either sex weighing between 150 - 200 grams were randomly selected from central animal facility, JSS Medical College, Mysore excluding diseased and pregnant rats. Animals were housed in groups of 3 at an ambient temperature of $25 \pm 1^{\circ}\text{C}$ with *ad libitum* access to food and water. The study protocol was approved by Institutional Animal Ethics Committee.

Study design

In this study, 12 albino rats were used. Animals were randomly divided into 2 groups of 6 rats each; Group-1 (Control) received distilled water (1 ml/rat). Group-2 (Test) received flunarizine dissolved in distilled water (0.8 mg/kg body weight/day) for 5 days. All the rats were orally fed with the respective drug using a gavage tube. On the 5th day, 90 minutes following the last dose of the drug, all the rats were subjected to the OGTT. The capillary blood glucose levels (CBG) of all the rats were measured at 0 hour. After this, all the rats were given glucose (2 g/kg body weight) dissolved in water orally using gavage tube. Following this, the CBG of the blood sample from tail vein (obtained by tail snipping) was estimated at 60 and 150 minutes. CBG measurements were done by using standardized glucometer (ACCUCHEK).

Oral glucose tolerance test (OGTT)^[20]

OGTT is a measure of the glucose induced insulin secretion mediated glycemic alteration. The standard method of OGTT for normal rats with some modifications were done to assess the effect of flunarizine on glucose induced glycaemic control alteration.

The current study was performed to assess the effect of flunarizine on glucose induced glycemic control alteration.

Statistical Analysis

The data is presented by calculating the mean and SEM of the outcome parameters. One way Analysis of Variance (ANOVA), repeated measures ANOVA and independent samples T-test were applied to see the difference between two groups and the CBG levels at various durations of the OGTT of both the groups when required. Percentages and cross tabulations were done wherever it was found to be appropriate. Tests of significance were carried out at 5% level.

RESULTS

As shown in Table 1 / Figure 1, the mean CBG level of flunarizine group rats were higher at all times of OGTT i.e. 0, 60, 150 minutes from the time of administration of glucose compared to the control group and at 0 and 150 minutes the hyperglycemia induced in the flunarizine group was statistically significant compared to the control group ($p < 0.05$). The maximum increase of CBG levels in the flunarizine group were seen at 0 minutes which was 26% higher than the control group, followed by at 150 minutes which was 20.9% and at 60 minutes which was 7.03% higher compared to the control group.

The magnitude of increase in CBG levels induced by the test drug is only 18.6%

less compared to control at 0 - 60 min, extent of reduction in increased CBG levels induced by the test drug was 27.58 % less compared to control at 60 -150 min and increased CBG levels induced by the test drug was same as (100%) control at 0 - 150 min (Table 2). Though CBG levels were higher in the flunarizine group compared to the control group throughout the OGTT, the changes in CBG levels between different time intervals of OGTT was lesser (but not statistically significant) in the test group than the control group.

DISCUSSION

In this study, it was observed that flunarizine adversely affected the blood glucose levels at the beginning and the 2½ hour interval of OGTT and thus indicating its effect on basal blood glucose levels itself which is controlled by basal insulin release. It however caused lesser effect on peak glucose levels indicated by blood glucose levels at 60 min interval of OGTT. Even though the CBG levels were higher in flunarizine group compared to control group, the difference was statistically insignificant. This demonstrated an adverse effect of flunarizine on basal insulin release more than glucose induced insulin release.

The hyperglycemia at 0 min of OGTT was more with flunarizine group compared to the control which indicates that flunarizine inhibited the basal insulin release (Table 1 and 2). The same phenomenon was seen at 150 min

Table 1: Capillary blood glucose (CBG) levels in control group, flunarizine group, difference between control and flunarizine groups in mg/dl and percentage.

Time interval of OGTT	Blood glucose concentration in mg/dl (mean \pm SEM)			% increase in CBG levels in Flunarizine group	p value
	Control group (n = 6)	Flunarizine Group (n = 6)	Raise in flunarizine group compared to control group		
0 min	54.5 \pm 1.962	68.67 \pm 2.376	14.17 \pm 0.8333*	26	0.001
60 min	94.83 \pm 4.586	101.5 \pm 2.405	06.67 \pm 2.679	7.03	0.226
150 min	67.67 \pm 2.860	81.83 \pm 4.347	14.16 \pm 3.049 [†]	20.9	0.021

* $p < 0.05$, flunarizine vs control at 0 min

[†] $p < 0.05$, flunarizine vs control at 150 min

Table 2: Change in CBG of the groups between various time intervals of OGTT

Time interval	Change in CBG values (mg/dl)	
	Control	Flunarizine
0-60 min (1 hour)	40.33 ± 6.28	32.83 ± 4.38
60-150min (1 ½ hours)	27.17 ± 6.09	19.67 ± 5.83
0-150 min (2 ½ hours)	13.17 ± 1.89	13.17 ± 3.22

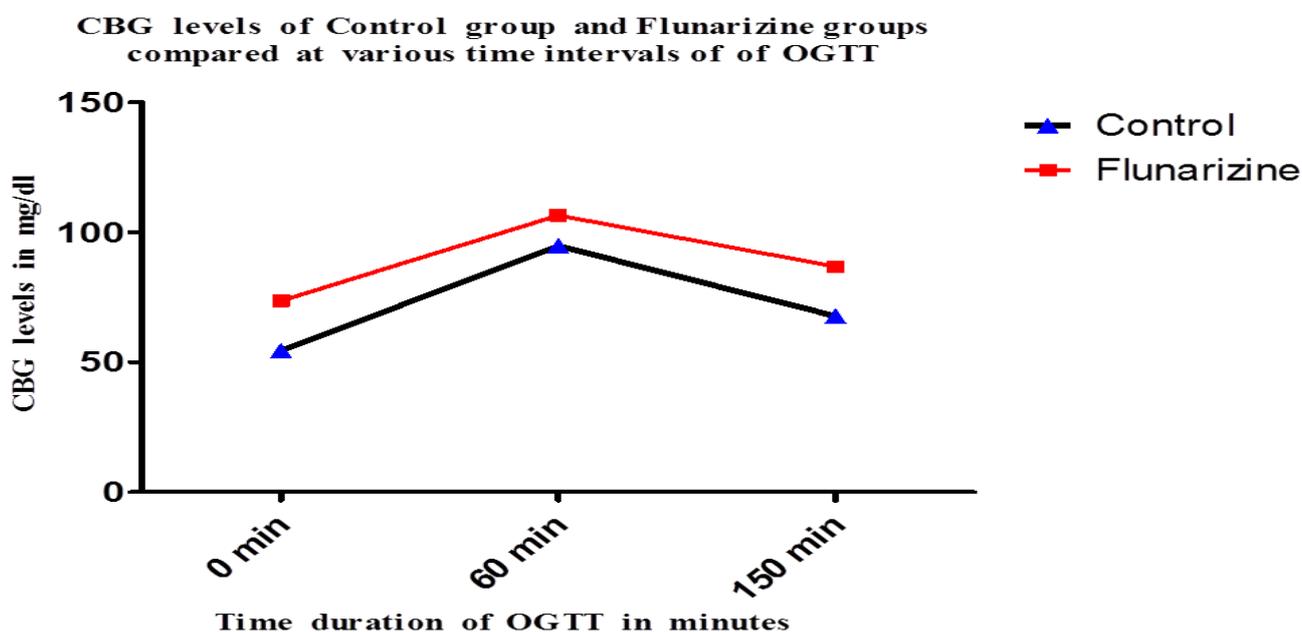
also re-establishing the above concept that flunarizine continued to exhibit hyperglycemic action even after the glucose challenged insulin release was over. But, the glucose challenge induced insulin release was incompletely inhibited at 60 minutes of OGTT which brings back the concept of differential blockade of T, N, L-type of calcium channels of beta cells by flunarizine since the type and extent of calcium channels active in the beta cells at basal insulin and glucose induced insulin release are known to be different.

The lesser quantum of hyperglycemic effect between 0 to 60 minutes of OGTT with the test drug is due to mild hyperglycemia seen even at 0 hour of OGTT with flunarizine compared to control which indicates that

flunarizine had shown inhibition of basal insulin release at 0 hour of glucose challenge itself (Table 1).

The lesser fall of CBG level between 60 to 150 minutes (Table 2) of flunarizine compared to the control is because of the pre-existing mild hyperglycemic action even at 0 hour of OGTT with flunarizine compared to control group which can be explained by the biphasic insulin secretion effect produced by the L-type channel which is blocked by flunarizine. This again highlights the concept of differential blockade of calcium channels of the pancreatic beta cells by flunarizine which concurs with the idea^[24] about the difference in the extent of blockade of various voltage gated calcium channels in cardiac and other tissues of the body by the various calcium channel blockers.

The level of inhibition of basal insulin release by flunarizine seems to be almost same at the beginning (0 min) and the end (150 min) of the OGTT reflected by the almost similar percentage of worsening of hyperglycemia at these intervals but a lesser inhibitory effect on glucose stimulated insulin release at 60 minutes evidenced by lesser worsening of

Figure 1: The CBG levels of control and flunarizine groups at 0, 60 and 150 minutes of OGTT are compared

hyperglycemia at 60 min of OGTT (Table 1). This milder effect of flunarizine in causing hyperglycemia at 60 minutes of OGTT may be hypothesized that the glucose stimulated insulin release which is the major control on blood glucose levels at 60 minutes of OGTT is mediated by the rapid acting and long lasting effect of L-type Ca^{2+} channels mainly. The L-type Ca^{2+} channels exhibit biphasic insulin secretion effects compared to the shorter acting T-type Ca^{2+} channels which have most of its effect on basal insulin secretion modulation.^[7]

The CBG differences at 2½ hours remain as high as at the 0 minutes of the OGTT thus indicating the persistent effect of flunarizine when given orally daily for a period of 5 successive days even though the time of peak plasma concentration of the stat oral dose is only 1-2 h. This gives an indirect evidence that the inhibition of insulin release by flunarizine persists with a daily dosing for 5 days irrespective of the of glucose challenge which is highlighted by the hyperglycemia even after glucose challenge is completed (150 minutes of OGTT).

From the above observations, it can be concluded that, when given for 5 consecutive days orally, flunarizine causes hyperglycemia by inhibiting basal insulin release to a large extent compared to a lesser extent of inhibition of glucose induced insulin release. Thus, the implication are, when flunarizine is being used in migraine patients with diabetes, regular monitoring of blood sugar levels and readjustment of the antidiabetic medications may be necessary.

Also, when patients with migraine are prediabetics / high risk diabetics, flunarizine may cause development of overt diabetes either by adding to an already existing insulin deficiency or insulin resistance.

This persistent effect of flunarizine for upto 21 days ($t_{1/2}$ is 19 - 21 days) even after stopping its administration should be

considered before estimating the blood glucose levels of patients with diabetes mellitus and migraine who are on treatment with antidiabetic drugs in addition to flunarizine because the hyperglycemia seen in such patients might be due to the persistent effect of flunarizine, which may be falsely attributed to poor glycaemic control by antidiabetic medications if the blood glucose is estimated within 21 days of the last dose of flunarizine. These findings are thus a sound of warning when flunarizine needs to be used over a long period of time in diabetics and prediabetics.

Another interesting outcome of this study is the possibility of the hyperglycemia induced by flunarizine being contributory to its effectiveness as a prophylactic drug in migraine, as migraine incidence is found to be lower in diabetics^[3] where hyperglycemia is the main metabolic abnormality. This is just the hypothesis generated by the findings of this study when considered in conjunction with the available knowledge about diabetes and migraine.^[3-5]

To conclude, flunarizine has hyperglycemic effects in normal albino rats when given for 5 consecutive days orally at the dose used for prophylaxis of migraine in human beings. Its hyperglycemic effect seems to be because of its property of blocking various types of calcium channels involved in the process of insulin release from pancreatic beta cells with maximal effect on basal insulin release than on glucose challenged insulin release.

Thus, when contemplating the use of flunarizine for prophylaxis in migraine therapy in a patient with prediabetes / high risk diabetes, it is advisable to be cautious about worsening of glycaemic status and its associated complications. The findings of this study encourages further research on the extent of blockade of the different calcium channels of the pancreatic beta cells by flunarizine and its consequent effects on insulin release

and on the role calcium channels of the pancreas as a target for antidiabetic therapy.

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