To evaluate the effect of pyridostigmine on blood glucose levels in euglycemic albino rats through OGTT

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Abstract

Introduction: The effect of Pyridostigmine on blood glucose levels in euglycemic albino rats through OGTT

Methods: Twelve Swiss albino rats weighing around 150-200gmsof either sex were randomly selected from the central animal facility, and divided into two groups. The control group received distilled water (25ml/kg body wt.), test group received Pyridostigmine (3.6mg/kg/day) for 5 days. All the drugs were given per orally. On the fifth day, following overnight fasting, 1 hour after drug administration OGTT was performed, by administering oral glucose in dose of 0.6gm/kg body weight. The capillary blood glucose level of both the groups were measured at 0, 60 and 150 minutes, by rat tail snipping method using (ACCUCHEK) glucometer.

Results: The Capillary Blood Glucose levels of Pyridostigmine group was less when compared to control group at all-time intervals and was statistically significant when compared to control at all the time intervals.

Conclusion: Pyridostigmine showed the hypoglycemic activity when given for 5 days orally in euglycemic albino rats through OGTT

Keywords: Oral Glucose Tolerance Test (OGTT), Capillary blood glucose, Diabetes, Pyridostigmine, Euglycemic.

Introduction

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular and neurological abnormalities. Diabetes has now become a high profile public health concern in its own way just not as earlier, due to the escalating epidemic of diabetes in older people and emergence of diabetes in children also, which is considered primarily as a risk factor for heart disease. Over the past 30 years, diabetes is one of the major causes of morbidity and mortality affecting the youth and middle aged people next to cardiovascular diseases.

The two broad categories of DM designated are type 1 DM and type 2 DM. Type 1 DM is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production and is often preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). [(1)]All these lead to both morbidity and mortality in these patients as a result of microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke, peripheral vascular disease) complications and leading to end organ damage.[(2)]

The common anti diabetic drugs used to control blood sugar level and also to minimize complication are Sulfonylureas, Biguanides, Meglitinides, Thiazolidinediones, alpha-glucosidase inhibitors, amylin analogues, GLP analogues, dipeptidyl-peptidase-4 inhibitors and Sodium Glucose Co-transporter 2 Inhibitors (SGLT-2) which can only be used in patients with type 2 diabetes. They are mainly used to replace the insulin deficiency by increasing insulin secretion from β cells or to enhance the action of insulin or decrease the insulin resistance which are the primary targets of the treatment of diabetes and its complications.[(3)]

After better understanding the pathophysiology of diabetes and importance of tight glycemic control to minimize the complication of diabetes leads to better modalities of treatment options with newer drugs or newer uses of existing approved drugs are being approached for research. In addition to this the newer upcoming post marketing surveillance SAE reports of existing antidiabetic drugs makes to set a goal to find out better options of management of diabetes if available.

Pyridostigmine is used in the treatment of myasthenia gravis, reversal of nondepolarizing muscle relaxants and pretreatment for soman nerve gas exposure.[(4)]

The acetylcholine/vagus effects on pancreatic insulin release are mediated by activation of muscarinic acetylcholine receptors located on the pancreatic β-cells.

M3 receptors are present in visceral smooth muscles, iris, ciliary muscle, exocrine glands, endocrine glands and vascular endothelium.[(5)]

They are G_q –protein coupled and activate the membrane bound phospholipase (PLC) generating inositol triphosphate (IP3) and diacylglycerol(DAG)

which in turn release Ca^{2+} intracellularly causing depolarization

Pyridostigmine is an anticholinesterase which inhibits metabolism of acetylcholine by acetyl cholinesterase thereby enhancing its cholinergic effects facilitating impulses across myoneural junction.(6)

Pyridostigmine has half-life of 1-2 hour and metabolized by liver into 3- hydroxyl- N – methylpyridinium. Pyridostigmine is eliminated through urine.(7)

Oral Glucose Tolerance Test (OGTT): -The oral glucose tolerance test is a measure of the glucose induced insulin secretion and its mediated glycemic changes. This study used OGTT for normoglycemic rats with some modifications to the standard method (Duvigneaud and Karr, 1925) to assess the effect of Pyridostigmine on glucose induced glycemic alteration.(8)

Thus the mechanism, by which anti cholinesterase acts to cause insulin secretion is,

1. Amplifying the triggering pathway by activation of Phospholipase C, which generates IP3 and diacylglycerol, a potent PKC activator.
2. Acetylcholine accelerates depolarization of plasma membrane of β cells by a Na^+ influx or by nonspecific cationic-dependent mechanism.

Both the above mechanism increases the insulin release and there by decrease the blood glucose level.

Hypothesis: Acetylcholine acting through M3 receptors, by activation of phospholipase C generates IP3 and diacylglycerol. It also depolarizes the membrane of insulin stored granules by sodium channel and causes secretion of insulin leading to decrease in blood glucose. Pyridostigmine an anti-cholinesterase, is hypothesized to exhibit the same activity.

Materials and Methods

The study was conducted after the approval of IAEC (Institution Animal Ethical Committee).CPSEA approval number from IAEC of: JSSMC/IAEC/12/5655/DEC 2013

Inclusion Criteria:

- Animals weighing 150-250gms.
- Age 3-4 months.
- Healthy with normal behaviour and activity.

Exclusion Criteria:

- Animals weighing more than 250gms and less than 150gms.
- Age < 3 months and >4 months.
- Pregnant rats and those which have delivered.

Animals used in the present study were adult healthy albino rats, of Wistar albino strain, weighing between 150-250gm of either sex. The rats were inbred in the central animal house, under suitable conditions of housing, temperature, ventilation and nutrition. The animals were fed with commercial laboratory food and water ad libitum. They were maintained at a temperature of 24-27^{0}c with relative humidity of 30-70 % with 12 hr light dark cycle. The animals were divided into 2 groups and 12 rats were used in total for the study. The doses of drugs were based on the human daily dose converted to that of animal dose using the standard formula.(9)

Drugs and chemicals: Tab. Pyridostigmine 60mg (SUN pharmaceuticals, India) was dissolved in distilled water and immediately administered orally, distilled water given orally, 0.6mg/kg of glucose mixed in distilled water for OGTT.

Methodology

The rats were divided into 2 groups containing 6 animals (n=6) in each group [control and test group].The test drug was given Pyridostigmine 2.5mg/kg/day orally and the control group was given distilled water 25ml/kg/day orally for 5 days.

Group 1:-Distilled water- 25ml/kg/day (orally)

Group 2:- Pyridostigmine 2.5mg/kg/day (orally)

All rats were fasted overnight before the 5th day. On the 5th day 1 hour after the last dose of the respective drug, OGTT was performed. All the rats were given glucose (0.6gm/kg body weight) orally using gavage tube. Following this, the Capillary blood glucose (obtained by tail snipping) was assessed at 0, 60, and 150 minutes of time intervals using a glucometer (ACCUCHEK).

Statistical Analysis

The results have been statistically analyzed for significance by calculating the Mean values, Standard Deviation, the t-Test using one way analysis of variance (ANOVA) at different time intervals within the same group followed by independent sample t-test between the two groups. Results were presented as Mean ± SD. For all the tests a ‘P’ value of 0.05 or less was considered for statistical significance. All the statistical analysis was done by using IBM SPSS 21 software.

Results

Pyridostigmine group showed fall in capillary blood glucose levels throughout the OGTT compared to control with the maximum fall at 60 min.

Table 1: Capillary Blood glucose (CBG) levels in control, and Pyridostigmine group and the difference between the control and Pyridostigmine group at corresponding time intervals.

Time interval during OGTT	Capillary Blood glucose concentration in mg/dl		
	Control group N=6	Pyridostigmine group N=6	Fall in CBG levels (Difference between the control and Pyridostigmine group)
0 min	73.16 ±4.87	65.5±2.88 *	7.66±1.99
60 min	100.33±3.55	82.33±3.66 **	18±0.11
150 min	82±4.19	71.5±4.59 *	10.5±0.4

Data is expressed as mean ± SD of n=6, *p<0.05 and** p< 0.01compared with control (distilled water). SD: Standard Deviation

The Pyridostigmine group showed fall in CBG levels when compared to control at all-time intervals, with maximum fall at 60 min.Thus, the fall in CBG levels of Pyridostigmine was statistically significant (p<0.05) at 0 min, 60 min and 150 min

Table 2: Percentage fall in Capillary Blood glucose (CBG) level in Pyridostigmine group when compared to control

	0 min	60 min	150 min
Pyridostigmine	10.47	17.94	12.8

Data are expressed in percentage

Table 3: The difference in the CBG levels of Pyridostigmine and control group at time intervals 0 min, 60 min and 150 min of OGTT

S. No	Time interval	Change in CBG values	
		Control	Pyridostigmine
1	0-60 min	27.17±1.32	16.83±0.78*
2	60-150 min	18.33±0.64	10.83±0.93*
3	0-150 min	8.84±0.68	6±1.71*

Data is expressed as mg/dl, *p<0.05 compared with control (distilled water)

The Capillary blood glucose (CBG) levels of Pyridostigmine when compared to control was lower at all-time intervals, the CBG level inter interval differences of Pyridostigmine i.e., 0-60 min, 60-150 min and 0-150 min is lower when compared to control and is statistically significant(p<0.05).

Table 4: Depicts difference in CBG values between control group and Pyridostigmine group at various time intervals

S. No.	OGTT time interval of Control - OGTT time interval of Pyridostigmine (C- T)	Difference in CBG values (mg/dl)
1	0-0 min	7.66*
2	0-60 min	9.17
3	0-150 min	1.66 *
4	60-0 min	34.83*
5	60-60 min	18 *
6	60- 150 min	28.83*
7	150-0 min	16.5*
8	150-60 min	0.33*
9	150-150 min	10.5*

Data is expressed as mg/dl, *p<0.05 compared with control (distilled water)

Discussion

Diabetes mellitus is defined as a metabolic disease characterized by hyperglycemia, due to defects in insulin secretion , insulin sensitivity, environmental factors, genetic factors, stress and many more, often affecting blood vessels, heart ,kidney, eye, foot etc. In the recent past many hypoglycemic agents were introduced but still the diabetes and the related complications continue to be a major medical problem not only in developed countries but also in developing countries, because just control of hyperglycemia is not sufficient to manage diabetes. About 20% of the world's adult population have the metabolic syndrome, of them majority are diabetic with insulin resistance, in which the major components are blood glucose levels and lipid levels. Diabetics are twice as likely to die of heart attacks or cardiovascular accidents and three times as likely to have a heart attack or stroke compared with people without metabolic syndrome.

Normally, the insulin release from β cells has two phases. The first/early phase begins within minutes of a glycemic stimulus. Early-phase insulin primes tissues that are sensitive to it, in particular liver, which results in the reduction of hepatic glucose output. Type 2 diabetes is characterized by insufficient insulin secretion by pancreatic β cells and reduced peripheral sensitivity to the effects of insulin (insulin resistance). In type 2 diabetic patients the important defect in insulin secretion is the impairment of early-phase insulin release which is both delayed and blunted.[10]

In pancreatic β ells, glucose stimulates insulin secretion by generating triggering and amplifying signals. The triggering involves the following sequence of events: entry of glucose by facilitated diffusion, metabolism of glucose by oxidative glycolysis, rise in the ATP-to-ADP ratio, closure of ATP-sensitive K+ (KATP) channels, membrane depolarization, opening of voltage-operated Ca2+ channels, Ca2+ influx, rise in cytoplasmic free Ca2+ concentration ([Ca2+]i), and activation of the exocytotic machinery. Under these conditions, glucose still increases insulin secretion in a concentration-dependent manner. The amplification pathway consists of an increase in efficacy of Ca2+ on exocytosis of insulin granules. There exists a clear hierarchy between both pathways. The triggering pathway predominates over the amplifying pathway, which remains functionally silent as long as [Ca2+]i

has not been raised by the first pathway; i.e., as long as glucose has not reached its threshold concentration. The amplifying pathway serves to optimize the secretory response not only to glucose but also to non-glucose (amino acids) stimuli. In Type 2 diabetes the amplifying pathway is impaired. The available drugs act on K^+ATP channels and increase the triggering signal, novel drugs that correct a deficient amplifying pathway would be useful to restore adequate insulin secretion in type 2 diabetic patients.[11,12]

In the present study Table 1 and Table 2, Pyridostigmine group showed decrease in the Capillary blood glucose(CBG) levels at all-time intervals of OGTT i.e.,0 min, 60 min, and 150 min when compared to control. The CBG level at 0 min is 7.66±1.99more when compared to control i.e., 10.47%, which indicates indirectly, that Pyridostigmine increased basal secretion of insulin. The CBG level at 60 min is 18±0.11more when compared to control i.e., 17.94% which indicates that Pyridostigmine causes more glucose dependent insulin secretion from pancreatic β cells .The CBG level at 150 min is 10.5±0.4 more when compared to control i.e., 12.8% because of sustained action of Pyridostigmine on pancreatic β cells. Graph 1 showed the Capillary Blood glucose (CBG) levels and difference between control, and Pyridostigmine group. Graph 2 and Graph 3 showed the Reduction in CBG levels and Percentage fall in Capillary Blood glucose (CBG) of Pyridostigmine group compared to control group.

In Table 3,The inter interval difference of Pyridostigmine group at 0-60 min is maximum, which indicates glucose dependent insulin release and inter-interval difference at 60-150 min of Pyridostigmine group is more compared to 0-150 min because of sustained effect on pancreatic β cell. The interval difference at 0-150 min is the total combined effect of Pyridostigmine on pancreatic β cells. Graph 4 showed the difference in the CBG levels of Pyridostigmine and control group at time intervals 0 min, 60 min and 150 min of OGTT. Graph 5 showed difference in CBG values between control group and Pyridostigmine group at various time intervals.

The above findings indicate that Pyridostigmine acts as a hypoglycemic drug in normal albino rats. OGTT test is used to assess the glucose tolerance which indirectly indicates the insulin sensitivity and beta cell function. Hence, in diabetics and pre-diabetics it can be assumed that Pyridostigmine causes decreased in blood glucose levels.

Conclusion

The test drug Pyridostigmine showed significant decrease in capillary blood glucose level in euglycemic albino rats when compared to that of control through OGTT. The hypoglycemic activity of Pyridostigmine was maximum during the 60 min, which justifies the hypothesis stated above and enhances the glucose dependent insulin release.

Thus, to conclude Pyridostigmine causes decrease in blood glucose levels in euglycemic albino rats through muscarinic receptor stimulation, through activation of phospholipase C generates IP3 and diacylglycerol and also depolarizes membrane by sodium channel and causes secretion of insulin. Therefore decrease in blood glucose.

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Conflict of Interest: Nil

References

1. Alvin C Powers. Diabetes mellitus. Longo, Fauci, Kasper, Hauser, Jameson, Loscalzo. Harrison's principles of internal medicine. Mc Graw Hill. New York.2012.2152-2180.
2. Edwin Gerald, Siddheshwar Balakrishnan joshi, Dharam Chandra Jain. Diabetes and herbal medicine. Iranian journal of pharmacology and therapeutics. January2008;7(1):97-106.
3. Nicholson G and Hall G.M, Diabetes mellitus: new drugs for a new epidemic. British Journal of Anaesthesia 2011; 107(1):65–73.
4. Hilal-Dandan R. Muscarinic receptor agonist and antagonist. In: Bruton LL, editor. Goodman and Gilman's the Pharmacological Basis of Therapeutics. 12th ed. China: McGraw Hill;2011.p.311-25.
5. D. Gautam, S J Han, A Duttaroy, D Mears, F Hamdan, J H Li, et.al. Role of the M_3 muscarinic acetylcholine receptor in β-cell function and glucose homeostasis. Diabetes, Obesity and Metabolism.2007;9(2):158–169.
6. 7. Daniela Billups, Brian Billups, R A John Challiss, and Stefan R. Nahorski. Modulation of Gq-Protein-Coupled Inositol Trisphosphate and Ca2 Signaling by the Membrane Potential. The Journal of Neuroscience. 2006Sept27;26(39):9983–9995.
7. KD Tripathi. Cholinergic system and drugs. In: KD Tripathi. Essentials of medical pharmacology. New Delhi: Jaypee;2013.p.105-112.
8. Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose Tolerance Test minimal Model Indexes of β-cell Function and Insulin Sensitivity. Diabetes.2001;50:150-58.
9. Bikash Medhi, Ajay Prakash. Introduction to experimental pharmacology. In: Bikash Medhi, editors. Practical manual of experimental and clinical pharmacology. New Delhi: Jaypee;2010.p.23-25.
10. Tuomilehto J. Point: a glucose tolerance test is important for clinical practice. Diabetes Care2002;25:1880– 1882
11. Jean-Claude Henquin. Triggering and Amplifying Pathways of Regulation of Insulin Secretion by Glucose. Diabetes.2000November49:1751-60.
12. 12. Mitsuhisa Komatsu, Yoshihiko Sato, Satoko Yamada, Keishi Yamauchi, Kiyoshi Hashizume, and Toru Aizawa. Triggering of Insulin Release by a Combination of cAMP Signal and Nutrients an ATP-Sensitive K Channel–Independent Phenomenon. DIABETES. 2002Feb5(1):29