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RESEARCH ARTICLE
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A STUDY TO EVALUATE THE EFFECTS OF PYRIDOSTIGMINE ON BLOOD GLUCOSE LEVELS IN DEXAMETHASONE INDUCED DIABETIC ALBINO RATS.

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a chronic and progressive disease. It is characterised by the presence of both insulin resistance and relative insulin deficiency. At present, the management of T2DM include diet, lifestyle modification and oral hypoglycaemic drugs. Acetylcholine stimulates the release of insulin and pancreatic polypeptide (PP) from pancreas. PP inhibits gastric emptying rate, decreases food intake and enhances insulin sensitivity. Pyridostigmine acts by increasing the availability of acetylcholine at the cholinergic receptors.

Methods: Thirty adult Wistar albino rats weighing 200-350g, of either sex were taken into study and divided into 5 groups of 6 animals each. 1.Normal control 2. Dexamethasone control 3. Pyridostigmine control 4. Metformin+Dexamethasone 5.Pyridostigmine+Dexamethasone. Distilled water was given per orally to group1 for 7 days and group2 for initial 4 days. Dexamethasone was given to group 2, 4 and 5 intraperitoneally for the last 3 days. Metformin to group 4 and pyridostigmine to group 3 and 5 were given per orally. Oral glucose tolerance test was performed on 5^{th} and 7^{th} day at 0, 60 and 120 min time interval using a glucometer.

Results: Both metformin and pyridostigmine showed fall in blood glucose levels. But metformin showed reduced levels at all the time intervals compared to pyridostigmine.

Conclusions: Although pyridostigmine showed hypoglycaemic activity in dexamethasone induced hyperglycaemia in rats but metformin showed better antihyperglycemic effect

Keywords: Hyperglycaemia, Pyridostigmine, Metformin

1. INTRODUCTION :

Type 2 diabetes mellitus (T2DM) is a long-term and progressive metabolic disorder with rapidly increasing prevalence. T2DM constitutes 90% of all the diabetes cases and characterized by the

presence of both the peripheral insulin resistance and relative insulin deficiency. The disorder is characterised by increased lipolysis, production of fatty acids, increased hepatic glucose output, and decreased peripheral uptake of glucose.[1]
It was estimated, in year 2013, worldwide, 382 million people aged 20–79 years were living with diabetes, 46% of them being undiagnosed. This number is expected to increase to 592 million by the year 2035. India plays host to a 65.1 million patients with diabetes, second only to China as per estimates in International Diabetes Federation Atlas 2013.[2]
The management of T2DM comprise of healthy diet and lifestyle changes, oral antidiabetic agents such as biguanides, sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, GLP-1 receptor agonists, DPP-4 inhibitors, amylin agonists, and insulin therapy. Endocannabinoid antagonists acting at the CB1 receptor have been effective in reducing food intake and improving glucose homeostasis.[3]
Pyridostigmine bromide (3-[[(dimethylamino)-carbonyl] oxy]-1-methylpyridinium bromide), being a reversible inhibitor of acetylcholinesterase, has been used in the treatment of myasthenia gravis, reversal of neuromuscular blockade, and in nerve gas (i.e, soman) poisoning. Pyridostigmine can also be used to treat paralytic ileus and urinary retention after abdominal surgery.[4-6]

Action of acetylcholine/ vagus in the body on pancreatic insulin release is mediated through activation of muscarinic receptors located on the pancreatic β-cells. Pancreatic polypeptide (PP) is produced by the F-cells of the pancreas after cholinergic receptor stimulation. PP inhibits gastric emptying time and gallbladder motility, decreases food intake and increases energy expenditure in the gastrointestinal tract. Altered circulating PP levels are reported in several states of glucose intolerance, such as T1DM and T2DM. PP increases insulin sensitivity and reduces insulin requirements in patients with long-standing T2DM and T3DM. T3DM category consists of diabetes in association with, or as a consequence of, acute and chronic pancreatitis, pancreatic trauma pancreatic resection, pancreatic tumours, pancreatic stones, hemochromatosis, cystic fibrosis and agenesis of pancreas caused due to chronic pancreatitis and pancreatic resection. PP is an important modulator of peripheral insulin action.[7,8]
Pyridostigmine produce excitatory effect on nerves leading to increased neural activity. Through its cholinergic stimulation it produces increased levels of PP.[9]

Pyridostigmine produces actions like that of acetylcholine by increasing availability of acetylcholine at these sites (anticholinesterase) and make acetylcholine to act on cholinergic receptors.[10]

Hence, Pyridostigmine might be useful as a meal medication in DM. It can control rise in blood sugar levels after food intake as add on therapy, if not effectively being controlled by post prandial treatment protocols.
Thus, the mechanism by which cholinomimetics act to cause insulin secretion is:
1) Activation of PLC (phospholipase C), which produces IP3 and diacylglycerol, a potent PKC (protein kinase C) activator.
2) Acetylcholine also produces depolarization of β-cell plasma membrane by a Na+ or nonspecific cationic-dependent mechanism.[11]

2. HYPOTHESIS: Acetylcholine act on M3 receptors leading to activation of phospholipase C, which generates IP3 and diacylglycerol. It also produces depolarization of the membrane of insulin stored granules by sodium channel and causes release of insulin, therefore reduces blood glucose levels. Pyridostigmine stimulates the release of PP which increases peripheral insulin sensitivity and reduces insulin requirements in patients with long-standing Diabetes.
Thus, the present study is focused to evaluate the effects of pyridostigmine on blood glucose levels in dexamethasone induced diabetic albino rats.
The objective of the study was to evaluate the effect of pyridostigmine on blood glucose levels in dexamethasone induced hyperglycaemia in albino rats.

METHODOLOGY:

The study was conducted after the approval of IAEC (Institutional Animal Ethics Committee) (Reg.no.115/1999/ CPCSEA).Adult albino wistar rats of either sex of average weight 150-300gms were procured from the Central Animal House of K.S.Hegde Medical Academy, Mangaluru. The study was done in Department of Pharmacology during the month of September 2017. The rats were divided into 5 groups of 6 animals each. They were housed in polypropylene cages and fed standard rodent chow and water *ad libitum* throughout the experiment. The animals were cared for according to the "Guide for the care and use of Laboratory Animals", published by the National Academy of Science. Animals were acclimatized under standard laboratory conditions at 25 ± 2°C, 50 ± 15 percent relative humidity (RH) and normal photoperiod (12 h light:dark cycle) for seven days. After acclimatization, on day 1, fasting blood glucose (FBG) is measured for all rats using tail vein blood. Doses and route of administration of the drugs is discussed below.

Procedure:

- Group 1: Normal control
- Group 2: Dexamethasone control
- Group 3: Pyridostigmine control
- Group 4: Metformin+Dexamethasone
- Group 5: Pyridostigmine+Dexamethasone

The drugs were administered to the rats as mentioned below. The doses of drugs were based on the human daily dose converted to that of animal dose using the standard formula.[12]

- Group 1: Distilled water (25ml/kg body weight) per orally for 7 days.
- Group 2: Distilled water (25ml/kg/day) per oral for initial 4 days and Dexamethasone 8mg/kg body weight intraperitoneal for the last 3 days
- Group 3: Pyridostigmine (2.5mg/kg/day) per orally for 7 days
- Group 4: Metformin (100mg/kg/day) per orally for initial 4 days
- Group 5: Pyridostigmine (2.5mg/kg/day) per orally for initial 4 days

After 4^{th} day, for 5^{th},6^{th} and 7^{th} day Dexamethasone 8mg/kg body weight was given intraperitoneal to group 4 and group 5 Animals.

Metformin was dissolved in normal saline and administered orally at a dose of 100 mg/kg body weight according to previous published studies.[13]
Pyridostigmine was also dissolved in normal saline and was administered orally at a dose of 2.5mg/kg/day.

Estimation of biochemical parameters: Fasting Blood Glucose (FBG) was measured on 1^{st}, 5^{th} and 7^{th} day and Oral Glucose Tolerance Test (OGTT) was performed on 5^{th}, and 7^{th} day
Oral Glucose Tolerance Test (OGTT): - The OGTT is a measure of the glucose induced insulin secretion and its mediated glycaemic changes.All rats will be fasted overnight and half an hour after the last dose of the respective drug, OGTT will be performed. All the rats will be given glucose (0.6gm/kg body weight) orally using the gavage tube. The Capillary blood glucose levels (obtained by tail flicking) were assessed at 0, 60, and 120 min of time intervals using a glucometer (ACCUCHEK).

STATISTICAL ANALYSIS: The results were noted in Excel sheet. The results were analyzed by calculating the mean values, standard deviation and repeated measures of ANOVA. $p<0.05$ was considered significant.

RESULTS &DISCUSSION:

Both metformin and pyridostigmine showed significant fall in CBG levels when compared to control groups at all intervals, with maximum fall at 60 min.

Glucose is the primary stimulus for insulin secretion in pancreatic β cells. This occurs by triggering and amplifying signals involved. The following sequence of various events are characterised: glucose entry by facilitated diffusion, metabolism of glucose by oxidative glycolysis which is coupled with ATP sensitive potassium channels, increase in the ATP/ADP ratio, closure of ATP-sensitive K+ channels, depolarization of membrane, opening of voltage-gated Ca2+ channels, influx of Ca2+ ions, rise in cytoplasmic free Ca2+ levels, and exocytosis of insulin granules.[14]

Glucose stimulates insulin secretion in a concentration-dependent manner. This slight increase in glucose (as little as 1–6 mmol/l) can induce insulin release which again requires glucose metabolism, activation of protein kinases A and C, and does not seem to implicate long-chain acyl-CoAs. Changes in adenine nucleotides may also be involved. The amplification of signals produces an increase in Ca2+ levels which causes exocytosis of insulin granules. There exists a clear relation between both pathways. The triggering pathway predominates over the amplifying pathway, which remains functionally silent if Ca2+ levels has not been raised by the threshold concentration of glucose. The amplifying pathway serves to optimize the secretory response not only to glucose but also to non-glucose (amino acids and fats) stimuli. It is impaired in β-cells of Type 2 diabetic patients. Besides the existing drugs which act on K+ sensitive 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.[15,16]

In normal individuals, the early phase insulin release from β cells occurs within minutes of a glycaemic stimulus. There is impaired early-phase insulin release (delayed and blunted).

In Impaired Glucose Tolerance (IGT), basal insulin secretion is normal but glucose-stimulated early and late phase insulin secretion and (peripheral) insulin sensitivity is reduced. The loss of early-phase insulin release during and after the food intake affects normal glucose homeostasis: hepatic glycogenolysis and gluconeogenesis are not inhibited sufficiently, and glucose uptake by muscle is insufficient. This leads to the postprandial hyperglycaemia observed in glucose-intolerant and type 2 diabetic patients. OGTT has been a mainstay in testing β cells function and influence of glucose on glucose stimulated insulin secretion which is depicted at the periphery as change in glucose levels in the blood. In the present study, in **(Table 1/ Figure 1)** Both metformin and pyridostigmine showed reduced CBG levels compared to control groups at all the time intervals i.e. 0min, 60min and 120min. At 0 min, Group 5 shows lower CBG levels compared to group 2 i.e. dexamethasone control which indicates that pyridostigmine has increased the secretion of basal insulin. Further fall in CBG was observed at 120min compared to dexamethasone control group which is due to sustained and longer action of pyridostigmine. CBG levels were significantly low in metformin groups compared to pyridostigmine groups at all the time intervals.

In conclusion, the test drug Pyridostigmine showed significant decrease in CBG levels in dexamethasone induced hyperglycaemia in wistar albino rats when compared to that of dexamethasone control through OGTT with fall maximum at the 60th min. Although the test drug pyridostigmine showed hypoglycaemic activity in dexamethasone induced hyperglycaemia in rats but metformin showed better antihyperglycemic effect.

TABLES:

Table 1: OGTT results on day 7

	0 min	60 min	120 min
group 1	90	124	101
group 2	127	145	128
group 3	98	127	107
group 4	94	117	104

group 5	118	131	116

Figure 1: OGTT results on day 7

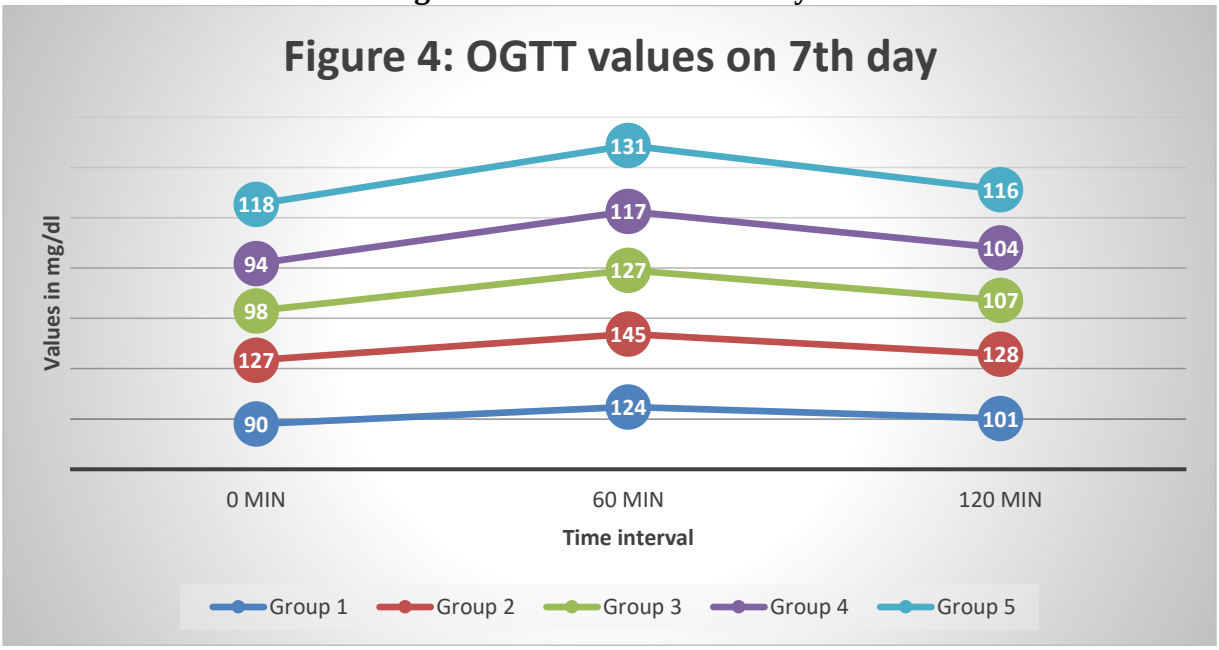

Table 2: ANOVA results

Group	Group	Mean difference	Std Deviation	Significance
1	2	-15.89	3.823	.003
	3	-2.83	3.823	.945
	4	6.75	3.823	.415
	5	-6.36	3.823	.473
2	1	15.89	3.823	.003
	3	13.06	3.823	.017
	4	22.64	3.823	.000
	5	9.53	3.823	.125
3	1	2.83	3.823	.945
	2	-13.06	3.823	.017
	4	9.58	3.823	.121
	5	-3.53	3.823	.885
4	1	-6.75	3.823	.415
	2	-22.64	3.823	.000
	3	-9.58	3.823	.121
	5	-13.11	3.823	.016
5	1	6.36	3.823	.473
	2	-9.53	3.823	.125
	3	3.53	3.823	.885
	4	13.11	3.823	.016

DECLARATIONS

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Conflict of interest: *None declared*

Ethical approval: *The study was approved by the Institutional Animal Ethics Committee*

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