EFFICACY AND SAFETY OF PROBIOTICS AS AN ADD ON THERAPY TO METFORMIN IN TYPE 2 DIABETES MELLITUS

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

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M.D. (PHARMACOLOGY)

BRANCH – VI

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, INDIA

APRIL 2017
CERTIFICATE

This is to certify that this dissertation entitled “Efficacy and safety of probiotics as an add on therapy to Metformin in Type 2 Diabetes Mellitus” by the candidate Dr.C.R.AnuRadha, for M.D. (Pharmacology) is a bonafide record of the research work done by her under the guidance of Dr. R.Jeyalalitha, M.D., Professor, Department of Pharmacology, Government Stanley Medical College, during the period of study (2014 - 2017), in the Department of Pharmacology, Government Stanley Medical College, Chennai 01.

I also certify that this dissertation is the result of the independent work on the part of the candidate.

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Diabetes mellitus is characterized by chronic hyperglycemia with associated disturbances in carbohydrate, fat and protein metabolism due to absolute or relative deficiency in insulin secretion and/or insulin action (1).

The worldwide diabetes mellitus incidence has been estimated to be around 285 million people in 2011, and this figure is expected to reach 438 million by 2030. The disease currently is found to occur in more than 7.1% of Indian adult population (2).

Age, sex and ethnic background are main risk factors for developing Type 2 DM (2). Diabetes Mellitus is the leading cause of blindness and end stage renal disease in adults aged 20 years (3).

The two major classifications of diabetes mellitus are Type 1 DM and Type 2 DM. Insulin deficiency causes Type 1 DM. Type 2 DM is characterized by insulin resistance, impaired insulin secretion, increased glucose production, and abnormal fat metabolism (4).

Chronic low grade inflammation and elevated levels of circulating pro-inflammatory cytokines are also features of insulin resistance and Type 2 Diabetes Mellitus (5).
## CONTENTS

<table>
<thead>
<tr>
<th>S.No</th>
<th>Title</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Review of literature</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Aim and Objectives</td>
<td>47</td>
</tr>
<tr>
<td>4.</td>
<td>Methodology</td>
<td>49</td>
</tr>
<tr>
<td>5.</td>
<td>Results</td>
<td>57</td>
</tr>
<tr>
<td>6.</td>
<td>Discussion</td>
<td>78</td>
</tr>
<tr>
<td>7.</td>
<td>Conclusion</td>
<td>81</td>
</tr>
<tr>
<td>8.</td>
<td>Bibliography</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Annexures</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
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<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
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<tr>
<td>DPP4</td>
<td>Dipeptidyl peptidase 4</td>
<td></td>
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<tr>
<td>SGLT</td>
<td>Sodium Glucose transporters</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
<td></td>
</tr>
<tr>
<td>GDM</td>
<td>Gestational Diabetes Mellitus</td>
<td></td>
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<tr>
<td>MODY</td>
<td>Maturity onset Diabetes of the young</td>
<td></td>
</tr>
<tr>
<td>HNF</td>
<td>Hepatocyte nuclear transcription factor</td>
<td></td>
</tr>
<tr>
<td>IPF</td>
<td>Insulin promoter factor</td>
<td></td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
<td></td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
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<tr>
<td>FPG</td>
<td>Fasting plasma glucose</td>
<td></td>
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<tr>
<td>OGT T</td>
<td>Oral Glucose tolerance test</td>
<td></td>
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<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
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</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
<td></td>
</tr>
<tr>
<td>SMBG</td>
<td>Self monitoring of blood glucose</td>
<td></td>
</tr>
<tr>
<td>SUR</td>
<td>Sulfonylurea receptor</td>
<td></td>
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<tr>
<td>PPAR</td>
<td>Peroxisome proliferator activated receptor</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<tr>
<td>ACTH</td>
<td>Adreno corticotropic hormone</td>
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</table>
US-FDA – United States Food and drug administration

HbA_{1C} – Glycated hemoglobin

GLP – Glucagon like polypeptide

GIP – Glucose–dependent insulinotropic polypeptide

CCK – Cholecystokinin

AMPK – Adenosine monophosphate activated protein kinase

OCT1 – Organic cation transporter 1

OCT2 – Organic cation transporter 2

LPS – Lipopolysaccharides

FFA – Free fatty acid

RBG – Random blood glucose

LDL – Low density lipoprotein
INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia with associated disturbances in carbohydrate, fat and protein metabolism due to absolute or relative deficiency in insulin secretion and insulin action.¹

The worldwide diabetes mellitus incidence has been expected to be around 285 million people in 2011 and this figure is expected to reach 438 million by 2030. The disease currently has been found to occur in more than 7.1% of Indian adult population.

Age, sex and ethnic background are the main risk factors for developing Type 2 DM.² Diabetes mellitus is the leading cause of blindness and end stage renal disease in adults aged 20-74 years.³

The two major classifications of diabetes mellitus are type 1 DM and type 2 DM. Insulin deficiency causes type 1 DM. type 2 DM is characterized by insulin resistance, impaired insulin secretion, increased glucose production and abnormal fat metabolism.⁴

Chronic low grade inflammation and elevated levels of circulating pro inflammatory cytokines are also features of insulin resistance and type 2 diabetes mellitus.⁵
Life style changes, high fatty diet restriction, daily exercise for 30 minutes five days a week are the gold standard preventive measures for diabetes. The major challenge in the treatment of diabetes is to control of raised blood glucose and associated symptoms along with prevention of complications and to improve quality of life and life expectancy.

Treatment modalities for Type 2 diabetes mellitus include oral anti diabetic agents and insulin in addition to life style modifications and diet. The oral anti diabetic agents available currently for therapy include insulin sensitizers and insulin secretagogues. Though many newer oral anti diabetic agents like DPP4 inhibitors, SGLT 2 inhibitors have been introduced, absolute glycemic control and arrest of progression of the disease is still not possible.

“Probiotics are live microorganisms when administered in adequate amounts confer a health benefit on the host”. Many types of bacteria are used as probiotics. Two common ones are lactobacillus and bifidobacterium.

Probiotics are primarily used to improve gastrointestinal disorder such as diarrhea, irritable bowel syndrome, constipation, lactose intolerance. In recent years several studies have suggested that probiotic could have beneficial effects in certain other disorders beyond gastrointestinal tract such as diabetes mellitus, hyperlipidemia, hypertension, obesity, eczema, psoriasis.
Recent Studies on probiotics have shown promising results in diabetes mellitus. Many studies have shown that probiotics may improve insulin resistance by reducing the inflammatory response in diabetes.\textsuperscript{12} Probiotics improve the blood glucose level by inhibiting the mediators like reactive oxygen metabolites and cytokines production that are responsible for the destruction of pancreatic cells.\textsuperscript{13}

In addition probiotics are associated with fewer side effects, in comparison to the other anti diabetic agents. This study has been undertaken with the aim of comparing the efficacy of probiotics as an add on therapy to metformin in type 2 DM.
REVIEW OF LITERATURE

DEFINITION OF DIABETES MELLITUS

Diabetes Mellitus is a common metabolic disorder that share phenotype of hyperglycaemia. Many factors contribute for hyperglycaemia including decreased insulin secretion and decreased utilization of glucose with increased production of glucose.\textsuperscript{14}

EPIDEMIOLOGY

It has been estimated that 285 million people had diabetes as of 2010. In 2030 this number is estimated to almost double.\textsuperscript{15} Though type 1 & 2 diabetes occur worldwide, type 2 DM is more common due to obesity and reduced physical activity. Diabetes is the fifth leading cause of death worldwide according to the recent estimate.\textsuperscript{16}

Type 1 DM is an autoimmune disorder that is common in childhood and early adulthood. Type 1 DM occurs in 5\% to 10\% and type 2 DM occurs in 90\% of all cases of diabetes mellitus. Overall prevalence of type 2 DM is 9.6\% in persons aged around 20 years.\textsuperscript{17} Among the chronic non communicable diseases, the mortality due to diabetes is 3.5\% .\textsuperscript{18}
Worldwide Prevalence of Diabetes Mellitus

Indian Scenario

Diabetes Mellitus currently affects 7.1% of Indian adult population. India is the diabetes capital of the world according to the International diabetes federation with 109 million individuals who will manifest diabetes by 2035. Additionally, a study by the American Diabetes Association reports that India will see the greatest increase in people diagnosed with diabetes by 2030.¹⁹
HISTORY OF DIABETES MELLITUS

Diabetes is described as “too great emptying of urine” from 1500BC. Indians Sushruta and Chakra identified Type 1 and Type 2 DM in 400 to 500 BC. In 1674 Thomas Willis, an anatomist physician discovered that the urine of diabetes individuals was sweet. In 1776, Matthew Dobson of Liverpool demonstrated that persons with diabetes excrete sugar in urine. In 1815, French chemist Michael Chevreula diagnosed that the sugar in diabetic urine was glucose.

First rational approach to the dietary treatment was devised by John Rollo. In 1914 to 1919 analytic methods for the glucose measurement in the urine and serum was devised. The terms for the common clinical forms of the diabetes such as diabetes maigre -diabetes of thin and diabetes grass diabetes of the fat were introduced by Lancereaux. Insulin was discovered in 1921 by Frederick Banting, John Macleod, and Charles Best. The first clinical trial of Insulin took place on 11th January 1922, on 14 year old Leonard Thompson. The first oral hypoglycemic agents suitable for clinical use were the sulfonylureas. Other oral agents soon followed with the biguanide metformin in 1960. In 1980 alpha glucosidase inhibitors became widely used. In 1990 thiazolidinediones were introduced.
CLASSIFICATION OF DIABETES

The two broad categories of DM are type 1 and type 2 diabetes. The classification is on the basis of the pathogenicity that leads to hyperglycemia.

PATHOGENESIS OF TYPE 2 DM

Triple abnormalities in the genesis of hyperglycemia in Type 2 DM are 1) Impaired pancreatic Insulin secretion 2) Peripheral resistance to insulin action 3) Excessive hepatic glucose output.27

Other abnormalities in diabetes are increased lipolysis, deficiency and resistance of incretin hormone, and hyperglucagonemia.28
GENETIC CONSIDERATION

Type 2 DM has monogenic and polygenic forms genetically. There is an increase risk of diabetes if the parents of individual both have type 2 DM.29

Monogenic forms of diabetes:

Forms with insulin resistance

- Mutation in the insulin receptor gene
  - Type A insulin resistance
  - Leprechaunism
  - Rabson Mendenhall syndrome
- Lipoatrophic diabetes
- Mutations in the PPARγ gene

Forms with defective insulin secretion

- Mutations in insulin, proinsulin genes.
- Mitochondrial gene mutations.
- Maturity onset diabetes of the young.
Resistance to the effects of insulin on glucose uptake, storage or metabolism is known as insulin resistance. It is the best predictor for progression of DM. Abnormalities of the insulin signaling pathway that occur in insulin resistance individuals are insulin receptors down regulations, decreased phosphorylation of insulin receptor and defect in translocation, fusion and docking of GLUT 4 containing vesicles with the plasma membrane.
IMPAIRED INSULIN SECRETION

The normal fasting insulin level is between 5 and 15µg/ml. It is elevated (>15µg/ml) in insulin resistant subjects. Puberty, pregnancy, sedentary lifestyle and weight gain are the common factors that lead to increased secretory burden on beta cells.

Insulin secretory abnormalities in type 2 DM

The following factors are responsible for the abnormalities in insulin secretory pattern of type 2 DM:

- Decreased glucose sensing
- Reduced or absent secretory response of insulin to oral glucose
- Alterations in the oscillations of insulin secretion
- Reduced effect of gastrointestinal hormones in potentiating glucose mediated insulin secretion.
- Inadequate insulin secretion for the magnitude of hyperglycemia.

EXCESS HEPATIC GLUCOSE PRODUCTION

In type 2 DM, due to insulin resistance there is increase in fasting blood glucose and decrease in storage of glycogen at the postprandial state. Glucose is produced by glycogenolysis of stored glycogen and gluconeogenesis from two and three carbon substrates derived primarily from skeletal muscle. Glucose
production of liver is primarily regulated by the relative actions of glucagon and insulin to activate or suppress glucose production respectively.\textsuperscript{34}

\textbf{In Type 1 and 2 DM, other specific types of DM and GDM the spectrum of normal glucose tolerance, prediabetes followed by diabetes is depicted above}

In gestational diabetes mellitus after delivery, raised blood glucose level returns to normal.\textsuperscript{35}
**RISK FACTORS**

- Family History (Parents with diabetes)
- Obesity (Body mass Index >25kg/m²)
- Habitual physical inactivity
- Race/Ethnicity (e.g. Pacific Islander, African American, Asian)
- Individuals with HbA1c of 5.7-6.4% or impaired glucose tolerance
- Hypertension (blood pressure >140/90)
- HDL less than 35mg/dl, TG level more than 250mg/dl
- History of gestational diabetes (delivery of baby weight more than 4kg)
- History of vascular disease
- Association of acanthosis nigricans, polycystic ovary disease.

**COMPLICATIONS OF DM**

**Acute complications:**

- Hyperglycemic hyper osmolar state
- Diabetic ketoacidosis
Chronic complications:

Microvascular

- Retinopathy (Non proliferative)
- Nephropathy
- Neuropathy

Macrovascular

- Coronary heart disease
- Peripheral arterial disease
- Cerebrovascular disease

Other complications

- Gastrointestinal
- Genitourinary
- Dermatological complications
- Infections
- Glaucoma
- Cataract
SCREENING OF TYPE 2 DM

Screening for type 2 DM recommended at every 3 years. In individual with risk factors must be screened at early age and frequently. The fasting plasma glucose is recommended screening test for type 2 diabetes. When a high index of suspicion for the disease is present glucose tolerance test (GTT) can be done in addition to fasting plasma glucose or alternatively.\textsuperscript{39}

PREVENTION OF TYPE 2 DM

Life style intervention and weight loss can reduce 30% to 60% of progression to diabetes.\textsuperscript{40} Metformin is useful for modest reduction in the progression of diabetes. Treatment of other cardiovascular risk factors should be done to prevent complications.

According to ADA recommendations “high risk individuals for progression to diabetes (age <60 years, BMI 35 kg/m\textsuperscript{2}, family history of diabetes, elevated triglycerides, reduced HDL, hypertension, or HbA1c>6.0%) metformin should be considered for prevention”. Individuals with HbA1c of 5.7–6.4% or impaired glucose tolerance have to be monitored annually.
CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS

- “Symptoms of diabetes with fasting plasma glucose 7.0 mmol/L (126 mg/dL) or
- Random blood glucose concentration 11.1 mmol/L (200mg/dl)
- Two-hour plasma glucose 11.1 mmol/L (200 mg/dL) during oral glucose tolerance test or
- HbA1C >6.5%” are the diagnostic criteria for the diabetes.

SYMPTOMS AND SIGNS

Polyuria and polydipsia, polyphagia, weight loss are the main symptoms of type 2 DM. In obese patients hyperglycemia is insidious and usually noted during routine laboratory studies.41

TREATMENT GOALS FOR DIABETES MELLITUS:

The goals of therapy for type 1 or type 2 DM are to eliminate symptoms related to hyperglycemia, to reduce the long term micro and macro vascular complications of diabetes and to allow patient to achieve a normal lifestyle.42
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<tr>
<td>HbA1c</td>
<td>&lt;7.0%</td>
</tr>
<tr>
<td>Preprandial-capillary</td>
<td>3.9–7.2 mmol/L (70–130 mg/dL)</td>
</tr>
<tr>
<td>plasma glucose</td>
<td>&lt;10.0 &lt;1.7 mmol/L (&lt;180 mg/dL)</td>
</tr>
<tr>
<td>Peak- postprandial capillary plasma glucose</td>
<td></td>
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<tr>
<td>Triglycerides</td>
<td>&lt;1.7 mmol/L (150 mg/dL)</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>&lt;2.6 mmol/L (100 mg/dL)</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>&gt;1 mmol/L (40 mg/dL) in men</td>
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<td></td>
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<td></td>
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<tr>
<td>Blood pressure</td>
<td>&lt;130/80</td>
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*Treatment goals for adults with diabetes are tabulated in above table.*

**TREATMENT OF TYPE 2 DIABETES MELLITUS**

The various modalities of treatment available for type 2 diabetes mellitus are life style intervention, patient education, medical nutrition therapy, exercise and pharmacotherapy.

**Lifestyle intervention**

Medical nutrition counseling, comprehensive diabetes education and exercise recommendations are the components of lifestyle intervention. There are many clinical trial evidences suggesting that proper administration of life style intervention lead to improved outcome.⁴³
**Patient education**

Diabetes self management education is providing knowledge to perform self care, make lifestyle changes. More frequent contact between the diabetic management team and the diabetic patient improves the control of glycaemia.

**Self monitoring of blood glucose (SMBG)**

Self monitoring of blood glucose is component of patient education. SMBG helps to reduce complications. The frequency of recommended screening test in diabetes therapy depends on the nature of the diabetes, overall treatment plan and the patient’s abilities. Patients are advised to monitor at least once a day as well as with hypoglycemic symptoms.

**Medical nutrition therapy**

Proper caloric intake along with other aspects of diabetes therapy is important for management of diabetes.

- **Nutritional recommendations for diabetes**

  **Weight loss diet**

  - low-fat or low-carbohydrate

  **Fat in diet**

  - Reduce trans fat consumption
Carbohydrate in diet

- Monitor carbohydrate intake in regards to calories
- Sucrose-containing foods may be consumed with adjustments in insulin dose
- Amount of carbohydrate determined by estimating grams of carbohydrate in diet
- Glycemic index reflects how consumption of a particular food affects the blood glucose

Other components

- Non nutrient sweeteners
- Usual supplements of, antioxidants, vitamins, trace elements are not advised

Exercise\textsuperscript{47}

To improve the insulin sensitivity and glycemic control the patient must do aerobic physical activity for 150 minutes per week. Exercise must be regular at least every 48 hours. Formal exercise tolerance test is vital in individual with duration >15 years-type 1 DM, >10 yrs -type 2 DM, age >35 years, retinopathy, microalbuminuria, nephropathy. Due to occurrence of retinal detachment or vitreous hemorrhage, untreated proliferative retinopathy is a contraindication to severe exercise.\textsuperscript{48}
Exercise training can delay the onset of type 2 DM in those at high risk. Exercise cause multiple benefits like reduced blood pressure, maintenance of muscle mass, reduction in body fat, reduction of cardiovascular risk, and weight loss.

PHARMACOTHERAPY

The various pharmacotherapeutic modalities available for treatment of DM include oral antidiabetic drugs and the various insulin preparations and analogues.

CLASSIFICATIONS

I. Insulin preparations and insulin analogues

II. Oral antidiabetic agents

Sulfonylureas

- **First generations**
  - Tolbutamide, Chlorpropamide
- **Second Generations**
  - Glibenclamide, Glipizide, Gliclazide, Glimepride, Meglitinides

- Repaglinide, Nateglinide

Biguanides

- Metformin
Thiazolidinediones

- Rosiglitazone, Pioglitazone

Alpha-glucosidase Inhibitors

- Acarbose, Meglitol

Other Drugs

GLP-1 Agonist

- Exenatide, Liraglutide

Dipeptidyl Peptidase-4 Inhibitors

- Saxagliptin, Sitagliptin, Vildagliptin

Amylinomimetics

- Pramlintide

Bile acid-binding resins

- Colesevelam

Bromocriptine

**ORAL ANTIDIABETIC DRUG**

**Sulfonylureas**

The sulfonylureas drugs have remained by far the most popular treatment for type2 diabetes throughout the world. Sulfonylureas act by stimulating insulin release from the beta cells of pancreas and decrease hepatic clearance of hormone and increase insulin level.
There are 2 groups of sulfonylureas. The first generation sulfonylureas Tolbutamide, Chlorpropamide. The second generation sulfonylureas are glyburide, glipizide and glimepiride. First generation sulfonylureas have a long half life, frequent side effects and greater frequency of hypoglycemia. Second generation sulfonylureas have shorter half life, rapid onset of action, more coverage of post prandial blood glucose.52

Sulfonylureas bind to the SUR1 subunit and block the ATP sensitive K⁺ Channel. Food and hyperglycemia reduces absorption of sulfonylureas. They are metabolized by liver and excreted in the urine. Sulfonylureas causes nausea, hypoglycemic reactions cholestatic jaundice, vomiting, agranulocytosis, hemolytic anemia, hypersensitivity reactions.
They are useful in controlling hyperglycemia in type 2 DM patient who cannot achieve control with diet changes. Beyond the endocrine pancreas they also block K+ channel on heart and prevent ischemic preconditioning.\

**Meglitinide analogues**

Meglitinide analogue acts by closing the ATP sensitive potassium channel by binding to the sulfonylurea receptor. The first member of this group is Repaglinide. It causes rapid pulse of insulin. Hypoglycemia is the major side effect. It also causes weight gain. It is metabolized by Cytochrome p450 3A4 enzyme. The drugs which induce or inhibit the CYP 3A4 enzyme increase or inhibit the meglitinide analogue metabolism.

**D phenylalanine derivative**

Nateglinide, d-phenylalanine derivative acts by stimulation of transient rapid release of insulin from beta cells by closure of the ATP sensitive K+ channel. In response to the intravenous glucose tolerance test it partially restores initial insulin release. It is mainly useful in individuals with isolated postprandial hyperglycemia. Dose titration is not required. Incidence of hypoglycemia is less with nateglinide.
**Biguanides**

Phenformin and metformin are the two biguanide group of antidiabetic drugs introduced in the 1950s. Phenformin was withdrawn and has been banned in India since 2003 due to high risk of lactic acidosis.\(^{56}\)

Biguanides act by decrease hepatic glucose production and increase insulin action on muscle and fat. It is an antihyperglycemic and not hypoglycemic. It is well absorbed and excreted in urine.

**Thiazolidinediones**

Thiazolidinediones are rosiglitazone, pioglitazone, and troglitazone. Troglitazone was withdrawn due to hepatotoxicity. They act by nuclear regulation of genes involved in glucose and lipid metabolism and adipocyte differentiation. Thiazolidinediones are the ligands of peroxisome proliferator activated receptor gamma (PPAR-\(\gamma\)). This is the part of the steroid and thyroid superfamily of nuclear receptor. Thiazolidinediones increase glucose transporter expression, decrease hepatic glucose output, decrease free fatty acid level and increase differentiation of preadipocytes to adipocytes.\(^{57}\)

Pioglitazone increases HDL and lowers triglycerides. Rosiglitazone increases total cholesterol, HDL cholesterol and LDL cholesterol. They have been shown to have a positive effect on endothelial function. Heart failure, loss
of bone mineral density, increase bone fractures, anaemia, and weight gain are the side effects of thiaglitazones group of drugs.

**Alpha glucosidase inhibitor**

α-glucosidase inhibitors inhibit the action of α Glucosidase enzyme in the intestinal brush border and reduce absorption of starch, dextrin and disaccharides. It slows the absorption of carbohydrates. Acarbose, miglitol and voglibose are the various types of α-Glucosidase inhibitors. They improve HbA1c level in severely hyperglycemic type 2 DM and reduce post prandial blood glucose in type 1 and type 2 DM.

Dose related malabsorption, flatulence, abdominal bloating and diarrhea are the gastrointestinal side effects caused by this drug. This can be reduced by titrating the dose of drug slowly.

**OTHERS**

**GLP 1 agonist**

Exenatide is a potent agonist at GLP 1 receptor which stimulates insulin secretions from beta cells of pancreas, decreases glucagon release, slows the rate of nutrient absorption, and decrease appetite. It only reduces post-meal glucose rise. Nausea, anorexia, diarrhea and necrotizing pancreatitis (rarely) are side effects of GLP1 agonist.\(^5\)
Dipeptidyl peptidase 4 inhibitors

Sitagliptin or Vildagliptin are selectively inhibiting DPP4 enzyme which inactivates GLP-1 which are orally active. They increase insulin secretion, decrease glucagon release, delay gastric emptying and suppress appetite. They are used mainly in resistant type 2 DM along sulfonylureas and metformin.59

Amylin analogue

Amylin is produced by pancreatic beta cells, reduces glucagon secretion, delays gastric emptying and promote satiety. Pramlintide is a synthetic amylin analogue which decreases postprandial hyperglycemia and exerts centrally mediated anorectic action.

Dopamine D2 agonist

Bromocriptine is approved as a adjunctive treatment of type 2 DM. It act on the dopaminergic control of circadian rhythm of hormone like GH, prolactin, ACTH release and reduce insulin resistance.60

Sodium glucose co-transport-2(SGLT 2) inhibitor

Sodium glucose transporter 2 accounts for 90% glucose reabsorption. SGLT-2 inhibition induces glucosuria and lowers blood glucose in type 2
Clinically approved SGLT2 inhibitors are Canaglifozin, dapaglifozin, and empaglifozin. Genital infections and urinary tract infections are the main side effects of SGLT2 inhibitors. In clinical trials there is an increase risk of breast cancer and bladder cancer due to dapaglifozin.  

**INSULIN IN TYPE 2 DIABETES**

Insulin is a small protein which contains 51 amino acids arranged in two chains (A and B) that are linked by disulfide bridges. The entire human pancreas contains 8 mg of insulin, approximately 200 biological units.

Stimulants for insulin secretions are glucose, mannose, gluconeogenic amino acids, high concentration of fatty acids, hormones such as GLP, glucagon, CCK, and sympathetic activity. The half-life of circulating insulin is 3-5 mins.

Insulin decreases glucose production by inhibiting glycogenolysis and suppresses gluconeogenesis. It also suppresses glucagon secretion by alpha cells of the pancreas and decreases FFA levels by decreasing lipolysis.

“Insulin preparations can be classified according to their duration of action into rapid, short, intermediate, and long acting and by their species of origin human or porcine”. Human insulin (HUMULIN, NOVOLIN) is now widely available as a result of its recombinant production. Porcine insulin differs from human insulin by one amino acid (alanine instead of threonine) at the carboxy terminal of the B chain, in position B30. The route of insulin
administration is subcutaneous injection using conventional disposable needles and syringes.

“Insulin preparations

- **Rapidly acting human insulin analogs**
  - Insulin lispro
  - Insulin aspart
  - Insulin glulisine
- **Short-acting regular insulin**
  - Regular insulin
- **Intermediate-acting insulins”**
  - NPH insulin
- **Premixed insulins**
  - 70% NPH/30% regular
  - 50% NPH/50% regular
  - 70% NPL/25% insulin lispro”
  - 50% NPL/50% insulin lispro
  - 70% insulin aspart protamine/30% insulin aspart
- **Long-acting human insulin analogs**
  - Insulin glargine
  - Insulin detemir”
30% of individuals with type 2 diabetes will benefit from insulin therapy for blood glucose control. When the combination of oral antidiabetic agents fails, the type 2DM patients require insulin.

When the RBG values are more than 250mg/dl particularly in lean individuals, in individual with renal or hepatic disease, or in individuals who are acutely ill or hospitalized insulin is considered as the initial therapy of type 2 DM.

**COMBINATION THERAPY IN TYPE 2 DIABETES**

Combinations of drugs are used for treatment of type 2 diabetes if glucose control does not attain therapeutic target. Addition of an insulin sensitizer with an oral insulin secretagogue provide good therapeutic results. When a combination of basal insulin with oral hypoglycemic drugs is given, the post prandial hyperglycemia is controlled by oral hypoglycemic drugs and fasting blood glucose levels are maintained by basal insulin.
METFORMIN

Metformin is biguanide group of antidiabetic drug. It was introduced in 1950. It is a white crystalline odorless powder with bitter taste and hygroscopic.

\[ \text{N,N-dimethylimidodicarbonimidic diamide hydrochloride.} \]

MECHANISM OF ACTION

Metformin acts by increasing the AMP dependent protein kinase activity. Activated AMPK stimulates glucose uptake, fatty acid oxidation, and non oxidative metabolism and reduces gluconeogenesis and lipogenesis.\(^{67}\) Activation of AMPK by Metformin is indirect by reducing intracellular energy stores.\(^{68}\)
Main action of metformin is antihyperglycemia rather than hypoglycemic effect. Metformin reduces blood glucose level by increasing the muscle glucose uptake and utilization, reduce the hepatic glucose production. It also delays the intestinal glucose absorption and increase the utilization of glucose by intestines, and erythrocytes that lead to increase lactate formation. Metformin reduces the fasting glucose, postprandial glucose and glycosylated hemoglobin level.

Metformin improves the lipid profile in diabetic and non diabetic persons who have hyperlipoproteinemia. It reduces the plasma triglycerides, total cholesterol and LDL cholesterol and increase the HDL cholesterol. Metformin retards intestinal absorption of glucose, other hexose aminoacids and vit B12.
PHARMACOKINETICS

Metformin is primarily absorbed from the small intestine. Oral availability is 50 to 60%. Concomitant food intakes slightly impair metformin absorption. Metformin accumulates mainly in wall of esophagus, stomach, kidneys, and duodenum and in salivary glands.

It does not bind to plasma protein and it is stable. OCT1 (Organic cation transporter 1) carry the metformin into hepatocytes and myocytes where it is pharmacologically active. OCT2 transport metformin into renal tubules for excretion. Elimination of Metformin is mainly by renal route. It is prolonged in patient with renal impairment.

DOSAGE OF METFORMIN

Metformin is available as an immediate release form. Currently recommended dosing is 0.5-1.0 g twice daily. A sustained release preparation is also available which is effective for once-daily dosing. Fixed dose combinations of metformin in combination with glipizide, glyburide, pioglitazone, repaglinide, rosiglitazone and sitagliptin are available.
THERAPEUTIC USE

It has been found to be of use in:

- Type 2 Diabetes (Non Insulin dependent diabetes)
- Obesity and insulin resistance
- Hyperlipoproteinemia

CONTRAINDICATIONS

- Acute complications like major trauma or operations
- Hepatic disorder
- Before X ray examinations with iodinated contrast materials
- Deficiency of vitamin B12, folic acid and iron.
- Ketosis prone diabetes
- Alcoholism
- Severe cardiovascular or respiratory disease.
- Diabetes with significant late complications

ADVERSE DRUG REACTIONS

Gastrointestinal adverse effects

- Nausea
- Diarrhea
- Indigestion
• Metallic taste
• Abdominal discomfort
• Anorexia.

GI adverse effects can be reduced by starting at lower dose and steadily titrating the dose and by taking the metformin with meals. Metformin is associated with lower blood levels of vitamin B12 due to malabsorption.\textsuperscript{74}

\textbf{Lactic acidosis} \textsuperscript{75}

Metformin provokes lactic acidosis due to increase lactate production or decreased elimination. Estimated incidence of lactic acidosis attributable to metformin use is 3-6 per 100,000 patient-years of treatment.\textsuperscript{76}

Nausea, vomiting, diarrhea and lower abdominal pains are symptoms of lactic acidosis. It should be confirmed by determination of plasma or blood lactate concentrations. Treatment of lactic acidosis includes bicarbonate infusion, glucose & insulin. Acute hemodialysis is the most definite treatment to correct the acidosis.

\textbf{Hypoglycemia}

When metformin is given alone hypoglycemia does not occur, but when metformin combined with sulfonylureas hypoglycemia will occur.
Others

Rarely Metformin induced skin reactions and hypersensitivity reactions can occur.

DRUG INTERACTIONS

• Cimetidine increases the metformin availability and reduces the clearance.\(^7^7\)

• Hyperglycemic drugs like thiazides, corticosteroids partly offset the antihyperglycemic action of metformin.

• Alcohol inhibits gluconeogenesis and potentiates the anti hyperglycemic and hyperlactemic effect of metformin.

• Acarbose and guar gum reduces absorption of metformin.\(^7^8\)
PROBIOTICS

The term probiotics derived from the Latin prefix pro which means for and the Greek noun βίος (bios) which means “life”.79

Probiotics are derived from the intestinal microbiota of healthy humans. “Antibiotics kill bacteria, but don’t discriminate between friendly and unfriendly organisms, so the balance between good and bad bacteria in the intestines can be upset”. But probiotics helps to restore the healthy balance of bacteria.80

The physiological effects of probiotics include synthesis of antibacterial substances, reduction of cholesterol level in the blood and stimulation of immune functions and the removal of carcinogens.81 Probiotics may be able to improve the lipid profile, which is the main risk factor for cardiovascular diseases.

GUTMICRBIOTA

At birth mammals have sterile gastrointestinal tract, and their microbiota is slowly accumulated by the physical contact with environment after birth.82 Infant’s intestinal microbiota is usually composed of enterobacteria bifidobacteria and it changes to more complex pattern at the adult stage.83
The human gastrointestinal tract contains about $10^{14}$ microorganisms/ml of luminal content. 90% of them are bacteroidetes phyla, mainly composed of Gram –ve bacteria, others belong to Fermicutes phyla composed of Gram +ve bacteria.  

Gut microbiota interacts at several levels in the function of the body. Intestinal microbiota composition is related with conditions such as allergies, intestinal inflammatory diseases cancer, cardiovascular disease, diabetes and dyslipidemia. The intestinal microbiota play vital role in many metabolic activities, like carbohydrate digestion, lipid metabolism, and glucose homeostasis.

**HISTORY OF PROBIOTICS**

Lactic acid bacteria were discovered by Pasteur in 1857. The therapeutic use of this bacteria was discovered by Ellie Metchnikoff. Bifidobacterium spp was discovered by Tissier. The first stable cultures of lactobacillus bacteria were prepared by Dr. Minoru Shirota in 1930.
DEFINITION

Probiotics are defined as “live microorganisms that when administered in adequate amounts confer a health benefit on the host”.

DIFFERENT TYPES OF PROBIOTICS

Probiotics are mainly strains of lactobacillus or bifidobacterium species which includes L. bulgaricus, L. casei, Lactobacillus GG, bifidobacterium bifidum, bifidobacterium longum, streptococcus thermophilus, enterococcus, and bacillus cereus.

CRITERIA FOR PROBIOTICS

Biological criteria for probiotics are acid tolerance, bile salt tolerance, beta galactosidase activity, antibiotic resistance, adherence to the gut mucosa, and antimicrobial potential. Probiotic have been used for the prevention and treatment of antibiotic associated diarrhea, acute infantile diarrhea and recurrent clostridium difficile infection.
Most common dietary sources of probiotics are yogurt, cheese, fermented milk, kefir, kimchi, ginger beer, miso, tempeh, sauerkraut, and soya beverages. Some fruit and vegetable juice products incorporated with live active micro organism cultures are good alternative for the lactose intolerant persons. Other forms of probiotics are supplements (tablets, pills or capsules) and powders.
STABILITY OF PROBIOTICS

The probiotic stability is due to many factors, which includes genus, species, type of strain and storage formulations. Probiotic cultures should be able to withstand processing conditions, and retain their probiotics properties after processing and they must survive in sufficient numbers in product. 

MECHANISM OF ACTION

Probiotics act by following mechanisms

- “Competition for dietary ingredients as growth substrates.
- Conversion of sugars into fermentation products which have inhibitory properties.
 ➢ Production of growth substrate such as exopolysaccharides or vitamins for the other bacteria.

 ➢ Direct antagonism by bacteriocins.

 ➢ Competitive exclusion for binding sites.

 ➢ Improved barrier function by stimulation key signaling pathways.

 ➢ Reduction of inflammation leads to alternation in the intestinal properties for colonization and persistence.

 ➢ Stimulation of inherent immune response and host antimicrobial peptides like defensins”.

 ➢ Immunomodulating the ability of pathogens to adhere or to invade colonic epithelial cells.\textsuperscript{91}

 ➢ Increasing the proinflammatory cytokine production.

 ➢ Altering the gene expression by pathogens.\textsuperscript{92}

 ➢ Release and possible distribution of antipathogenic factors.

**PHARMACOKINETICS**

 ➢ Adhesion to the intestinal epithelium

 The epithelial adhesion property differs between strains.\textsuperscript{93} This property is mainly needed for immunomodulatory activity of probiotics.\textsuperscript{94}

 ➢ Survival of Probiotics
Some micro organisms are damaged in stomach, others have high survival rate. Lactobacillus bulgaricus and Streptococcus thermophilus have a reduced resistance to acid, and they are destroyed within few minutes at pH 1 and about 1 hr at pH of 3.

- Colonisation of GIT by Probitics

Some probiotic strain preserve in feces for longer period because of colonization.

EXCRETION

Probiotics are excreted within few days after ingestion in feces at same rate or even quicker.

USES OF PROBIOTICS

- Antibiotic associated diarrhea
- Parasitic Infection
- Ulcer healing
- Cholesterol normalization
- Reduction in blood pressure
- Upper respiratory infection, Urinary tract Infection
- Improvement in vaginal health
- Reduce anxiety and depression
• Antioxidant property
• Prevent colon cancer
• Weight loss
• Diabetes Mellitus
• Coronary Heart Disease
• Lactose Metabolism & Food digestion
• Reduce Eczema

ADVERSE REACTION OF PROBIOTICS

Probiotics have been reported to produce only minimal or less adverse effects. Gasbloating, abdominal discomfort, flatulence, nausea, skin rashes, acute toxicity, and constipation are some of rarely reported side effects.

DRUG INTERACTIONS OF PROBIOTICS

Probiotic do not have any notable drug interaction. A laboratory study suggests that L. acidophilus fastens sulfasalazine metabolism.100
PRECAUTIONS

People with weakened immune systems must be cautioned while using probiotics.\textsuperscript{101} Because of the rare chance of bacterial infection people with artificial heart valves not recommended to take L. acidophilus.

INTESTINAL MICROBIOTA AND TYPE 2 DM

Increase inflammatory stress leads to insulin resistance and the interaction of intestinal microbiota with environmental factors and genetic factors, put in to the development of diabetes.\textsuperscript{102}

Altered intestinal microbiota cause increased intestinal permeability and mucosal immune response, that contribute to the development of diabetes.\textsuperscript{103}

Reduced tight junction proteins expression, favouring the bacterial lipopolysaccharide (LPS) translocation, that may result in metabolic endotoxemia and insulin resistance.\textsuperscript{104}

Type 2 diabetes is related with compositional changes in intestinal microbiota with significantly high proportion of bacteroidetes and proteobacteria compared to healthy individuals.\textsuperscript{105}

The lipopolysaccharides (LPS), main compound in gram-negative bacteria outer membranes are known as potent stimulators of inflammation and
inflammatory response, which exhibits endotoxaemia and play an important role in the development of diabetes.\textsuperscript{106}

The impaired balance of oxidants antioxidants may lead to cellular damages, insulin resistance and an enzymes dysfunction, along with occurrence of inflammation process and lipid peroxidation.

High fat diet, modify the intestinal microbiota, and increase the intestinal permeability which cause metabolic endotoxemia and insulin resistance.\textsuperscript{107}

Cani and co-workers hypothesized that gram negative bacteria present in the gut is connected with metabolic diseases, which explains differences between the diabetic and nondiabetic microbiota.\textsuperscript{108}

**ACTION OF PROBIOTICS IN TYPE 2 DM**

A number of reports confirmed experimentally and clinically that oxidative stress involved in the progression of diabetes mellitus as well as the subsequent complications.\textsuperscript{109}

“Generation of oxygen free radicals due to non-enzymatic protein glycosylation, auto-oxidation of glucose and impaired antioxidant defense enzymes are the most common consequences in diabetes”. L.acidophilus and L. casei attenuate oxidative stress and have antidiabetic effects. The antioxidative mechanisms of probiotics could be assigned to reactive oxygen species
scavenging, metal ion chelation, pro-oxidant enzyme inhibition and the reduction activity and inhibition of ascorbate oxidation.\textsuperscript{110}

Probiotics may improve insulin resistance by reducing the inflammatory response in diabetes. Probiotics improve the blood glucose level by inhibiting the mediators like reactive oxygen metabolites and cytokines production that are responsible for the destruction of pancreatic cells.
AIM

To determine the efficacy and safety of probiotics as an add on therapy to metformin in type 2 Diabetes Mellitus.

OBJECTIVES

- To study the efficacy of probiotics as an add on therapy with metformin in patients with type 2 diabetes mellitus in comparison to metformin monotherapy.
- To study the tolerability of probiotics in patients with type 2 diabetes.

END POINT

- Reduction in blood glucose level after 12wks (fasting blood glucose < 115mg/dl, postprandial blood sugar<140 mg/dl)
- Reduction of HbA1c level at the end of 12 weeks (<6.5%)
RATIONALE

Diabetes mellitus is the most common metabolic disorder. It produces microvascular and macrovascular complications. Among the chronic non-communicable diseases, the mortality due to diabetes is 3.5%.

Inspite of insulin and many oral anti-diabetic drugs available for treatment of type 2 DM, control of diabetes and its complications is still a major problem. Due to the side effects, these drugs cannot be continued for a long time and complete control of diabetes is not possible. Researches on various pharmacotherapeutic and alternative treatment modalities for treatment of diabetes exploring various areas of target are ongoing for better options.

Several human and animal studies have revealed that, the probiotic supplementation delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress.

Many Studies on probiotics have shown promising results in diabetes mellitus as a potential therapy towards the underlying inflammatory process associated with diabetes mellitus. Moreover probiotics are associated with less side effects. This study has been undertaken to study the efficacy and safety of probiotics.
METHODOLOGY

STUDY DESIGN

Single centre, prospective, randomized open label comparative study

STUDY CENTRE

Diabetology Outpatient Department,
Govt. Stanley medical college and Hospital, Chennai.

STUDY POPULATION

Patients with newly diagnosed type 2 diabetes mellitus

SAMPLE SIZE

100 patients (50 in control and, 50 in study group)

STUDY PERIOD

From August 2015 to August 2016

STUDY DURATION

12 weeks for each patient
STUDY DRUGS

- Cap.Probiotics (Lactobacillus sporogens 1 million, streptococcus fecalis 60 million, clostridium butyricum 4 million, bacillus mesentericus 2 million)
- Tab. Metformin 500 mg

DOSAGE FORMS USED

- Probiotics as capsule form, and metformin as tablet form
- Both the drugs were administered orally

SELECTION CRITERIA

➢ Inclusion criteria

✓ Both sexes.
✓ Age- 40 to 65 years.
✓ Newly diagnosed type 2 diabetes mellitus patients with
  - 8 hours fasting blood glucose level between 126 mg/dl - 140 mg/dl and/or
  - 2 hour plasma glucose level during a 75g of Oral glucose tolerance test between 200-240 mg/dl and
  - HbA1c level between 6.5-7%.
✓ Patient who are willing to give written informed consent and have no objection to participate in the study.

➤ **Exclusion criteria**

✓ Patients with type 1 diabetes mellitus.

✓ Patients with type 2 diabetes mellitus on other anti diabetic agents other than metformin.

✓ Patients with type 2 diabetes mellitus with any intercurrent illness.

✓ Patients with type 2 diabetes mellitus with complications of diabetes mellitus.

✓ Chronic gastrointestinal disorders.

✓ Patients taking antibiotics, antacids, H2-receptor blockers, proton pump inhibitors, loperamide, fuosemide, corticosteroids, cholestyramine.

✓ Co morbid conditions like renal, hepatic insufficiency and cardiovascular diseases.

✓ Chronic alcoholism.

✓ Pregnant & lactating women.

✓ Patients not willing to participate in the study.

**PROCEDURE**

The study was approved by the Institutional ethical committee of Govt. Stanley Medical College. The patients for this study were recruited from
the outpatient department of diabetology, Govt Stanley Medical College, Chennai.

Patients willing to participate in the study were included in the study. Details of the study were explained to the participants and the written informed consent was obtained in their regional language.

SCREENING

Screening procedure consisted of a detailed medical and drug history, thorough clinical examination followed by laboratory investigations which included fasting and postprandial blood glucose, HbA1C, and basic blood investigations.

After screening of 120 patients, 20 patients were excluded based on selection criteria. A total of 100 patients of both sexes and age between 40 to 65 years who fulfilled the inclusion criteria were recruited for the study.

RANDOMIZATION

The study subjects were randomly assigned using a computer generated randomization chart to either of the two groups as Group A and Group B, each group consisting of 50 patients.
GROUP ALLOCATION

Group A (Control group); Patients in this group were given T. Metformin 500 mg twice daily with meals for 12 weeks.

Group B (Study group); Patients in this group were given T. Metformin 500 mg twice daily with meals and Cap. Probiotics 1 capsule twice daily with meals for 12 weeks.

All patients were reviewed at the end of 2\textsuperscript{nd}, 4\textsuperscript{th}, 8\textsuperscript{th} and 12\textsuperscript{th} week. At each visit fasting and postprandial blood glucose were monitored. HbA1c was done at the end of 12\textsuperscript{th} week. Adverse event monitoring was also done throughout the study period.

ADVERSE EFFECTS MONITORING

Patients were monitored for adverse events at every visit. In addition patient were advised to do self monitoring of blood glucose whenever needed as per advise. If blood glucose value vary during SMBG the patients were instructed to report at diabetology department immediately.
FOLLOW UP

After 12 weeks of study period the patients were followed up in the diabetology department.

OUTCOME MEASURES

The efficacy measure was the reduction in the fasting blood glucose, postprandial blood glucose and HbA1c at the end of 12 weeks.

Safety evaluation was based on the spontaneously reported adverse events and changes in the laboratory values after the study.

STATISTICAL ANALYSIS

The results were analyzed statistically using SPSS software. Data was analysed as Mean ± standard deviation. Student independent t test was used for comparing quantitative data between the two groups and Chi square test was used for comparing qualitative data.

At the end of the study the effects of metformin alone and combination of probiotics and metformin in reduction of fasting, postprandial blood glucose and HbA1c was compared in terms of therapeutic efficacy and adverse effects.
SCREENING (120)

RECRUITMENT

INCLUDED (100)

GROUP A (50 pts) T. Metformin 500 mg BD for 12 weeks

GROUP B (50 pts) T. Metformin 500 mg BD +

REVIEW AT END OF 2, 4, 8 AND 12 WEEKS

DATA COMPILATION AND ANALYSIS

EXCLUDED (20)

- On other anti diabetic agents other than metformin-10
- Liver dysfunction-3
- Renal dysfunction-2
- Cardiovascular diseases-1
- Chronic alcoholism-4
RESULTS

Total of 120 diabetic patients were selected and screened for study. Based on the selection criteria, 20 patients were excluded and the remaining 100 patients allocated randomly into two groups of 50 patients each.

Baseline characteristics of both the groups including the mean age, sex distribution and basic blood investigations were assessed and tabulated.

Results of this comparative study was analysed statistically by student independent t test, pearson chi square test and paired t test.
Table 1: Age distribution

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean age in yrs</th>
<th>SD</th>
<th>Std. Error Mean</th>
<th>Student’s independent t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>51.34</td>
<td>6.00</td>
<td>0.858</td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>50</td>
<td>52.84</td>
<td>4.40</td>
<td>0.622</td>
<td>p=0.16</td>
</tr>
</tbody>
</table>

*P≤0.05 Significant, P>0.050 Not significant

**P≤0.01 Highly significant, ***P≤0.001 Very high significant,

Table 1 shows the age distribution between two groups. The mean age of patients in the control group was found to be 51.34yrs vs 52.84 in study group. There was no statistically significant difference between two groups (p=0.16).
Figure 1: shows the age distribution between two groups. The mean age of patients in the control group was found to be 51.34yrs vs 52.8 yrs in study group. There was no statistically significant difference between two groups.
Table 2: Sex distribution

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control</th>
<th>Study</th>
<th>Pearson Chi-square test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>56%</td>
<td>27</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>44%</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100%</td>
<td>50</td>
</tr>
</tbody>
</table>

*P≤0.05 Significant, P>0.05 Not significant, ** P≤0.01 Highly significant, ***P≤0.001 Very high significant,

Table 2: shows sex distribution of patient in both groups. The ratio of male to female in control group was found to be 28:22 and study group was 27:23. Statistical analysis was done by pearson chi square test. There was no significant difference in gender distribution between the two groups (P=0.305)
Figure 2(a) and (b) shows the gender distribution of the control and study group. The sex distribution of the patients were 52% male & 48% female in control group, 54% male and 46% female in study group. There was no significant difference in gender distribution between the two groups.
Table 3: Mean fasting blood glucose in both groups

<table>
<thead>
<tr>
<th>Fasting blood glucose</th>
<th>Control</th>
<th>Study</th>
<th>Student independent t test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Baseline</td>
<td>133.94</td>
<td>3.51</td>
<td>133.36</td>
</tr>
<tr>
<td>Visit 1</td>
<td>124.86</td>
<td>3.23</td>
<td>122.80</td>
</tr>
<tr>
<td>Visit 2</td>
<td>112.22</td>
<td>5.02</td>
<td>113.02</td>
</tr>
<tr>
<td>Visit 3</td>
<td>103.86</td>
<td>5.41</td>
<td>103.56</td>
</tr>
<tr>
<td>Visit 4</td>
<td>94.76</td>
<td>5.60</td>
<td>93.20</td>
</tr>
</tbody>
</table>

Within group p=0.001***

<p>| | | |</p>
<table>
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<tr>
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</tbody>
</table>

*P≤0.05 Significant, P>0.050 Not significant, ** P≤0.01 Highly significant, ***P≤0.001 Very high significant,

Table 3 shows comparison of mean fasting blood glucose values at weekly interval. Statistical analysis was done by using student independent t test.
Control group- Fasting blood glucose was significantly reduced from base line 133.94 to 94.76 at the end of 12 weeks (p=0.001)

Study group- Fasting blood glucose was significantly reduced from base line 133.36 to 93.2 at the end of 12 weeks (p=0.001)

There was no statistically significant difference between study and control group in reduction of fasting blood glucose at the end of 12 weeks (p=0.155)
Figure 3: Shows comparison of mean fasting blood glucose values between control and study group. There was no statistically significant difference between study and control group in reduction of fasting blood glucose at the end of 12 weeks (p=0.155).
Table 4: Percentage reduction in fasting blood glucose

<table>
<thead>
<tr>
<th>Fasting blood glucose reduction percentage</th>
<th>Control</th>
<th>Study</th>
<th>Student independent t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Visit 1</td>
<td>6.75</td>
<td>2.48</td>
<td>7.88</td>
</tr>
<tr>
<td>Visit 2</td>
<td>16.18</td>
<td>3.90</td>
<td>15.23</td>
</tr>
<tr>
<td>Visit 3</td>
<td>22.40</td>
<td>4.71</td>
<td>22.30</td>
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<tr>
<td>Visit 4</td>
<td>29.20</td>
<td>4.65</td>
<td>30.06</td>
</tr>
</tbody>
</table>

*P≤0.05 Significant, P>0.05 Not significant ,** P≤0.01 Highly Significant, ***P≤0.001 Very high Significant

Table 4 shows comparison of percentage reduction in fasting blood glucose.

Control group: 29.20% reduction of fasting blood glucose at the end of 12 weeks. Study group: 30.06% reduction of fasting blood glucose at the end of 12 weeks. There was no statistically significant difference in percentage reduction of fasting blood glucose between two groups.
Figure 4: shows comparison of percentage reduction in fasting blood glucose. There was no statistically significant difference in percentage reduction of fasting blood glucose between two groups.
Table 5: Mean post prandial blood glucose levels in both groups

<table>
<thead>
<tr>
<th>Post Prandial Blood Glucose</th>
<th>Control</th>
<th>Study</th>
<th>Student independent t test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Baseline</td>
<td>227.32</td>
<td>8.43</td>
<td>224.54</td>
</tr>
<tr>
<td>Visit 1</td>
<td>216.46</td>
<td>8.72</td>
<td>210.22</td>
</tr>
<tr>
<td>Visit 2</td>
<td>200.56</td>
<td>8.10</td>
<td>198.40</td>
</tr>
<tr>
<td>Visit 3</td>
<td>188.18</td>
<td>5.98</td>
<td>187.46</td>
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<tr>
<td>Visit 4</td>
<td>179.68</td>
<td>6.55</td>
<td>174.92</td>
</tr>
<tr>
<td>Within group p=0.001***</td>
<td></td>
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</tr>
<tr>
<td>Within group p=0.001***</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P≤0.05 Significant, p>0.05 Not significant  
** P≤0.01 Highly Significant, ***P≤0.001 Very high Significant,

Table 5: shows comparison of mean postprandial blood glucose values at weekly interval. Statistical analysis was done by using student independent t test.
Control group: postprandial blood glucose was significantly reduced from baseline 227.32 to 179.68 at the end of 12 weeks (p=0.001).

Study group: postprandial blood glucose was significantly reduced from baseline 224.54 to 174.92 at the end of 12 weeks (p=0.001).

There was no statistically significant difference between two groups in reduction of postprandial blood glucose level at the end of 12 weeks (p=0.07)
Figure 5: shows comparison of mean postprandial blood glucose between control and study group. There was no statistically significant difference between two groups in reduction of postprandial blood glucose level at the end of 12 weeks (p=0.07)
Table 6: Percentage reduction in post prandial blood glucose

<table>
<thead>
<tr>
<th>Post prandial blood glucose reduction Percentage</th>
<th>Control</th>
<th>Study</th>
<th>Student independent t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Visit 1</td>
<td>4.78</td>
<td>1.56</td>
<td>6.32</td>
</tr>
<tr>
<td>Visit 2</td>
<td>11.72</td>
<td>3.33</td>
<td>11.54</td>
</tr>
<tr>
<td>Visit 3</td>
<td>17.13</td>
<td>3.40</td>
<td>16.40</td>
</tr>
<tr>
<td>Visit 4</td>
<td>20.88</td>
<td>3.36</td>
<td>22.01</td>
</tr>
</tbody>
</table>

*P≤0.05 Significant, p>0.05 Not significant **P≤0.01 Highly Significant, ***P≤0.001Very high Significant,

Table 6: shows comparison of percentage reduction of post prandial blood glucose at weekly intervals. There was 20.88% reduction weeks in control group and 22.01% reduction in study group at the end of 12 weeks. There was no statistically significant difference between control and study group in percentage reduction of post prandial blood glucose(p=0.181)
Figure 6

Figure 6: shows comparison of percentage reduction in post prandial blood glucose between control and study group. There was no statistically significant difference between control and study group in percentage reduction of post prandial blood glucose. (p=0.181)
Table 7: HbA1c

<table>
<thead>
<tr>
<th>HbA1c</th>
<th>Control</th>
<th>Study</th>
<th>Student independent t-test</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Before study</td>
<td>6.75</td>
<td>0.11</td>
<td>6.69</td>
</tr>
<tr>
<td>After study</td>
<td>6.39</td>
<td>0.13</td>
<td>6.41</td>
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<tr>
<td>Within group</td>
<td></td>
<td></td>
<td>Within group</td>
</tr>
<tr>
<td>p=0.000***</td>
<td></td>
<td></td>
<td>p=0.000***</td>
</tr>
</tbody>
</table>

*P≤0.05 Significant, **P≤0.01 Highly Significant, ***P≤0.001 Very high Significant, p>0.05 Not significant

Table 7: shows comparison of mean reduction in HbA1c before and after study in both the groups. Control group showed a reduction in HbA1c from base line 6.75 to 6.39 at the end of 12 weeks(p=0.000). Study group showed a reduction in HbA1c from base line 6.69 to 6.41 at the end of 12 weeks(p=0.000). There was no statistically significant difference between control and study group in reduction of HbA1c (p=0.274)
Figure 7: shows comparison of mean reduction in HbA1c before and after study in both the groups. There was no statistically significant difference between control and study group in reduction of HbA1c (p=0.274).
Table 8: Adverse events

<table>
<thead>
<tr>
<th>S. No</th>
<th>Adverse events</th>
<th>Control group</th>
<th>Study group</th>
<th>Chi square test</th>
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<tbody>
<tr>
<td>1</td>
<td>Nausea</td>
<td>3 (6%)</td>
<td>0</td>
<td>P=0.04</td>
</tr>
<tr>
<td>2</td>
<td>Flatulence</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Abdominal discomfort</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Indigestion</td>
<td>2 (4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Diarrhea</td>
<td>6 (12%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Metallic taste</td>
<td>2 (4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>18 (36%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 8: shows the incidence of adverse events observed in both the groups during the study. 18 patients in the control group vs 3 patients in the study group experienced minor self limiting side effects.
Figure 8: shows incidence of adverse events in both groups. 18 patients in the control group vs 3 patients in the study group experienced minor self-limiting side effect. There was statistically significant difference between the groups (p=0.04) in occurrence of adverse events.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
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<th></th>
<th></th>
<th>Student paired t test</th>
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<td></td>
<td></td>
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<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>control</td>
<td>11.56</td>
<td>1.32</td>
<td>11.68</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>study</td>
<td>11.63</td>
<td>1.54</td>
<td>11.46</td>
<td>1.36</td>
</tr>
<tr>
<td>Total Count</td>
<td>control</td>
<td>8260.60</td>
<td>1931.85</td>
<td>8338.60</td>
<td>1812.68</td>
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<td>9013.80</td>
<td>2946.72</td>
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<td>2892.96</td>
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<tr>
<td>Urea</td>
<td>control</td>
<td>21.62</td>
<td>6.07</td>
<td>21.64</td>
<td>5.73</td>
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<tr>
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<td>5.32</td>
<td>19.78</td>
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<tr>
<td>S.Creatinine</td>
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<td>0.22</td>
<td>0.62</td>
<td>0.22</td>
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<tr>
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<td>0.65</td>
<td>0.15</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>control</td>
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<td>21.90</td>
<td>162.68</td>
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</tr>
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<td>4.86</td>
<td>44.84</td>
<td>4.39</td>
</tr>
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<td>3.76</td>
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<td>98.72</td>
<td>24.68</td>
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<tr>
<td></td>
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<td>15.53</td>
<td>111.86</td>
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</tr>
<tr>
<td>TGL</td>
<td>control</td>
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<td>25.57</td>
<td>160.52</td>
<td>24.60</td>
</tr>
<tr>
<td></td>
<td>study</td>
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<td>12.33</td>
<td>173.46</td>
<td>12.74</td>
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<td>Total Bilirubin</td>
<td>control</td>
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<td>0.20</td>
<td>0.46</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
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<td>0.34</td>
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<td>SGOT</td>
<td>control</td>
<td>22.80</td>
<td>5.63</td>
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</tr>
<tr>
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<td>21.90</td>
<td>4.11</td>
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<tr>
<td>SGPT</td>
<td>control</td>
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<td>5.96</td>
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<td>4.25</td>
<td>0.43</td>
<td>4.15</td>
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</tr>
</tbody>
</table>
Table 9: Shows the basic hematological investigations before and after the study in both the groups. Paired t test was used for analysis. There was no significant change between baseline and at the end of the study values in control and study groups.
DISCUSSION

Diabetes Mellitus is the most common non communicable disease. Insulin resistance, insulin secretion impairment, increased production of glucose lead to Type 2 Diabetes Mellitus. Type 2 Diabetes Mellitus is associated with many microvascular and macrovascular complications.

Life style modifications, exercise, medical nutrition therapy and pharmacotherapy including oral antidiabetic drugs and insulin are the various modalities of type 2 DM treatment.

Inspite of the various treatments available, tight control of Type 2 DM is still difficult due to the short comings of the available treatment. Therefore there has been a rising need to investigate and introduce innovative drugs which can be of potential use for adequate control of the disease.

Probiotics are live microorganisms which when administered in adequate amount confers a health benefit on the host. Lactobacillus and bifidobacterium are the most commonly used strains of probiotics.

In many animal and human studies it has been found that probiotics lowered blood glucose levels.
Matsuzaki \textsuperscript{111} in 1997 showed that oral administration of L. Casei in KK-A\textsuperscript{Y} mice decreased plasma glucose levels and inhibited the beta cell specific CD4 T cells and cytokines production which are the binding factor for induction of Diabetes Mellitus.

Tabuchi\textsuperscript{112} in 2003 reported that Lactobacillus rhamnosus GG improved glucose tolerance.

Yadav et al,\textsuperscript{113} in 2007 found that Lactobacillus acidophilus and Lactobacillus Casei significantly delay the onset of glucose intolerance, hyperglycemia, hyperinsulinemia and dyslipidemia.

Hariom et al,\textsuperscript{114} in 2007 found that probiotic supplementation reduced oxidative stress and hyperglycemia in fructose induced diabetes rats indicating lower risk of diabetes and its complications.

Al Salami et al,\textsuperscript{115} in 2008 showed that administration of probiotics can be a beneficial adjuvant therapy in type 2 Diabetes treatment.

Andreasen AS\textsuperscript{116}, et al (2010) proved that probiotics can reduce the inflammatory process which is needed for development of diabetes.

Ejtahed HS\textsuperscript{117} et al proved that lactobacillus and bifidobacterium noticeably reduce blood glucose levels, glycated Hb, and improve antioxidant status and total serum antioxidant capacity in type 2 diabetes patients.
With evidence from all these studies, this study was conducted to study the efficacy and safety of probiotics as an add on therapy to metformin in type 2 diabetes mellitus.

In our study the mean age of the patients in the control group was 51.34 yrs, 52.84 yrs in the study group.

The sex distribution of the patients were 52% male & 48% female in control group, 54% male and 46% female in study group. There was a male preponderance in both the groups.

Statistical analysis showed that both the groups to be comparable in terms of mean age, age distribution and sex distribution. Patients compliance was good in both the groups and follow up was good in both groups.

**Effect on Fasting Blood Glucose**

The mean fasting blood glucose was significantly reduced in both the control (p=0.001) and study group (p=0.001). But there was no statistically significant difference between both groups (p=0.155) in reduction of blood glucose.

**Effect on post prandial blood glucose**
The mean post prandial blood glucose was significantly reduced in control (p=0.001) and study group (0.001). But there was no statistically significant difference between both groups (p=0.07) in reduction of post prandial blood glucose.

**Effect on HbA1c**

HbA1c was significantly reduced in control (p=0.000) and study group (p=0.000). But there was no statistically significant difference between both groups (p=0.274) in reduction of HbA1c levels.

**Safety profile**

Probiotics did not significantly alter the haematological parameters such as Hb, total count, WBC count, renal and liver function tests. The study group was found to produce less adverse events in comparison to metformin monotherapy. The reported gastrointestinal side effects associated with metformin therapy were found to be less with the study group which was statistically significant (p=0.04). There were no serious adverse reactions in both groups.

The effect of combination therapy of probiotics with metformin 500 mg bd has produced a significant reduction in fasting, post prandial blood
glucose and HbA1c which is similar to control group with no statistically significant difference.

At present human study with monotherapy of probiotics is very limited as well there are no long term studies for effects of probiotics in patients with type 2 DM.

This study has been initiated to study the efficacy and safety of probiotics in patients with uncomplicated type 2 diabetes mellitus. Probiotics has been used as a combination therapy along with metformin for any additional beneficial effects over a period of 12 weeks.

From this study it can be concluded that probiotics has not produced any significant additional beneficial effects in combination therapy with respect to efficacy. The reported gastrointestinal side effects associated with metformin therapy were found to be less with the study group. The potential effect of probiotics in type 2 diabetic mellitus can be further investigated on a large scale population over a longer period of time.
CONCLUSION

Probiotics as an add on therapy with metformin compared to metformin monotherapy in type 2 diabetes mellitus has been found to reduce the fasting blood glucose, postprandial blood glucose and HbA1c levels, but the difference was not statistically significant between the groups.

From this study it is concluded that probiotics has not produced any significant additional beneficial effects in combination therapy with respect to efficacy. But the reported gastrointestinal side effects associated with metformin therapy were found to be less with the probiotics study group which was statistically significant. Therefore the potential effect of probiotics in type 2 diabetes mellitus can be further investigated on a large scale population over a longer period of time.
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Proforma : Case sheet

Name:

Age:

Sex:

Hospital No:

Complaints:

Present History:

Past History:

Personal History:

Family history:

Treatment History:

**General Examination:**

Vital signs:

Anaemia/Lymphadenopathy:

Pedal Edema/Jaundice:

Height/Weight/Waist circumference:

**Systemic Examination:**

CVS RS

ABDOMEN CNS
Drug form:

1. Name of patient:

2. Age:

3. Sex:

4. OP/IP No:

5. Address:

6. Drug Given:
   
   Batch No:
   
   Strip No:
   
   Date of purchase:
   
   Date of manufacturing:
   
   Date of expiry:

7. Drug Given:
   
   Batch No:
   
   Strip No:
   
   Date of purchase:
   
   Date of manufacturing:
   
   Date of expiry:
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</table>
கல முன்னெணும் பல்லவம்

................................................................. கலா முன்னெணும் பல்லவத்தியின் தலைமை விளக்கம் / தறிக்கை ................................................................. அவ்வூர் இந்த அரச முன்னெணும் பல்லவத்தின் பதிவு மற்றும் முன்னெணும் பல்லவத்தின் போர்வரை அதிகாரியான தனித்துவமான பாதுகாப்பு திறனோடு இந்த முன்னெணும் பல்லவத்தின் போர்வரை அதிகாரியான தனித்துவமான பாதுகாப்பு திறனோடு இந்த முன்னெணும் பல்லவத்தின் போர்வரை அதிகாரியான தனித்துவமான பாதுகாப்பு திறனோடு இந்த முன்னெணும் பல்லவத்தின் போர்வரை அதிகாரியான தனித்துவமான பாதுகாப்பு திறனோடு

இந்த அவ்வூர் முன்னெணும் பல்வேறு அரசாங்க அமைச்சுக் கூழ்பாட்டியின் கலா முன்னெணும் பல்லவத்தில்

இந்த அவ்வூர் 12 மாதங்கள் இடையில் இந்த அவ்வூர் முன்னெணும் பல்லவத்தின் கலா முன்னெணும் பல்லவத்தின் கலா முன்னெணும் பல்லவத்தின்

இந்த அவ்வூர் கலா முன்னெணும் பல்லவத்தில் மற்ற பதிவுகள் மற்றும் பதிவுகள்

இந்த அவ்வூர் கலா முன்னெணும் 

செயல்பாடுகளை செய்ய முன்னெணும் பல்வேறு அரசாங்கங்கள் செய்யப்பட்டு முன்னெணும் பல்வேறு அரசாங்கங்கள்

செயல்பாடுகளை செய்ய முன்னெணும் பல்வேறு அரசாங்கங்கள் செய்யப்பட்டு முன்னெணும் பல்வேறு அரசாங்கங்கள்


d: வகுப்பு:

தலாச: வகுப்பு}

சேவைப்பாடு:

இந்த: அரச முன்னெணும் பல்லவத்தின்
INFORMED CONSENT

I Mr/Mrs-----------------------. The aims, objectives, risks and possible adverse effects of the drugs used in this study, ‘Efficacy and safety of probiotics as an add on therapy to metformin in type 2 diabetes mellitus ’ as well as the importance of this study have been explained to me in a language I understand. I also understand that I will be required for the follow-up of this study and I will make myself available when required. However, I have the right to withdraw from the study at any time, without assigning any reason for my withdrawal.

SIGNATURE OF PATIENT                                    SIGNATURE OF DOCTOR

Name
Address
கைலாச பந்தவ நூல்

பொருளாதாரத்தில் அதிகாரத்தில் நாட்டுற்றல் செய்வது முற்படுகிறது. இத்தகைய நிலையில் நாட்டுற்றல் வாழ்வு செய்யும் குறுக்கு செயல்பாடுகளும் நீண்ட குறந்திகள் ஆகியவை அளக்கும் நூற்றாண்டு வரையாக அளவுத்தன. வருடம் வருடம் புரட்சிப் போர்களை நடத்தப்பட்டது.

பொருளாதாரத்தில் அதிகாரத்தில் நாட்டுற்றல் வாழ்வு செய்யும் சுருக்கம் ஆகியவை குறுக்கு செயல்பாடுகளும் நீண்ட குறந்திகள் ஆகியவை அளக்கும் நூற்றாண்டு வரையாக அளவுத்தன.

இது அப்பெயர் புரட்சிப்படுத்தப்பட்டது முதலில் அவ்வர் வருடம் ஒன்றுக்கும் நாட்டுற்றல் வாழ்வு செய்யினார். பின்னர் புரட்சிகள் வருடம் ஒன்றுக்கும் புரட்சிப்படுத்தப்படுவது. இது ஆண்டு 12 மாதங்கள் தோன்றியது. இது ஆப்பெயர் நேரப்பாடா நேராகப் புரட்சிகள் வைப்பது. இது நேர்த்து புரட்சிகளும் நாட்டுற்றலும் புரட்சிகளும் அளவுத்தன ஒன்று வரையாக.

இது ஆப்பெயர் விளக்கமாகப் புரட்சிகள் வைப்பது. நேர உணர்வுகூடு நிகழ்த்தும் போது அப்பெயர்பெயர்பெயர்பெயர்பெயர்

இது ஆப்பெயர் கட்டுமான நிகழ்த்தும் குற்றவங்களாக நடை அளவுத்தன விற்கூடு நிகழ்த்தும் வைப்பது. குறுக்கு செயல்பாடுகள்.

இது ஆப்பெயர் புரட்சிகள் வைப்பது புரட்சிகள் வைப்பது ஒன்றும் வரையாக அளவுத்தன. குறுக்கு செயல்பாடுகள் வைப்பது. பொருளாதாரமாகப் புரட்சிகள் வைப்பது ஒன்றும் வரையாக அளவுத்தன.
இந்த விளையாட்டுக்கூற்றில் இருக்கும் அவற்றின் தொடர்பு தன்மையாகக் கவனிக்க வேண்டும். அம்மாறு முன்னேற்றது பங்குகளின் காரணத்துடன் அந்தந்த கூற்று விளக்கும் திறனைப் பெற்றது.