***The Effect of***

***Plant Extracts***

***to Control
Hyperglycemia***

SCIENCE RESEARCH PROJECT

By

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**Chapter 1: Project Overview**

**1.1 Introduction**

Last summer, I observed my diabetic grandmother using cinnamon and fenugreek in her cooking. From ancient times certain herbs and spices have been used to treat diseases in Indian culture. She used these to reduce sugar in the blood.

Diabetes mellitus is a metabolic disorder of the Endrocrine System. Continuous use of the synthetic antidiabetic drugs can cause serious side effects. With an increasing health conscious population, seeking natural and non-toxic therapy is gaining attention. Different antidiabetic agents work differently - some stimulate more secretion of insulin and some work to reduce blood sugar. Bitter gourd stimulates pancreas to produce more insulin and plant extracts (cinnamon, fenugreek) reduce/delay production of glucose.

I wondered if these ancient medicinal plants could have potential use in diabetes treatment and could be a natural and low cost alternative (medicine) to regulate blood sugar, especially in developing countries.

**1.2 Background**

Diabetes is a lifelong chronic disease where high levels of glucose are found in the blood. There are two types of diabetes.

**Type 1:** The body doesn’t produce insulin and is treated with injecting insulin.

**Type 2:** The body either doesn’t produce enough insulin in the cells, or cells resist insulin. The high insulin level is unable to channel glucose into the muscles cells, and converts glucose into fats often resulting in heart disease.

In a healthy person, insulin helps turn food into energy in an efficient manner. The stomach breaks down carbohydrates from food into sugars, including glucose. Glucose then enters the bloodstream, which stimulates the pancreas to release insulin in just the right amount. Insulin, a hormone, allows glucose to enter cells throughout the body, where it is used as fuel. Excess glucose is stored in the liver.

In type 2 diabetes, the cells cannot absorb glucose properly. That means glucose levels in the blood become elevated. If one has developed a condition called insulin resistance, the body makes excess insulin, but the muscle, liver, and fat cells do not use or respond properly to the insulin. With long-standing uncontrolled type 2 diabetes, the pancreas will reduce the amount of insulin it produces.

The prevalence of diabetes has risen at an alarming rate. Considering the heterogeneity of diabetes and the limitations of current therapies, such as high secondary failure rates and significant side effects, there is an urgent need to explore novel health-promotion and therapeutic strategies. One intriguing approach to control diabetes could be its prevention and treatment by phytochemicals present in the diet that improve postprandial glycaemic control by Diet Induced Thermogenesis (DIT). DIT, caused by enzyme inhibition or heat generation in brown adipose tissue, is the additional calories the body burns from its internal reserves due to special diet intake. In Type 2 Diabetes, postprandial hyperglycemia, an exaggerated rise of blood sugar after a meal occurs due to chronic imbalance between energy intake and expenditure.

Postprandial hyperglycemia is related to the amount and digestion rate of consumed starch, which is the primary source of blood glucose. One important approach for treating postprandial hyperglycemia is to reduce or slow dietary carbohydrate digestion and absorption. This approach can be achieved by inhibiting starch hydrolysing enzymes in the digestive system. Mammalian starch digestion primarily occurs in the small intestine through the action of **α-amylase**, yielding both linear maltose and branched isomaltose oligosaccharides, which are further hydrolysed by **α-glucosidases** to release glucose. **Synthetic and naturally** derived compounds are known to reduce postprandial hyperglycemia by inhibiting key carbohydrate metabolizing enzyme in the small intestine such as **α-glucosidase**. For example, phenolic compounds comprised in plants have been found to be potent inhibitors of carbohydrate hydrolysing enzymes. In a screening test among more than 300 food extracts and compounds, it is observed that they exert significant inhibition of α-glucosidases, suggesting its potential use for diabetes management.

Centuries ago, Hippocrates, the father of medicine, observed that we should “**Let food be thy medicine and medicine be thy food.”** Today, those words are truer than ever as we become increasingly aware of conventional medicine’s limitation when it comes to treating acidity. There should be more clinical trials with natural remedies to prove their effectiveness to provide relief from various disorders.

**1.3 Problem Question**

Acarbose like drugs inhibits α-glucosidase and are responsible for reducing post-prandial hyperglycemia. As a result such medications are useful to persons who have been diagnosed with type 2 diabetes.

Can natural plant based agents like bitter guard, fenugreek, grapes, and cinnamon have similar effect on enzyme activity and work as potential inhibitors? The presence of polyphenols and flavonoids in fenugreek might be responsible for uncompetitive inhibitor. Vicine, charantin, and polypeptide-P present in bitter gourd might be responsible for its anti-diabetic properties. When a large number of compounds are to be tested, or when compounds are synthesized with minor modifications in functional groups or different percentages of extract/fractions, in vitro data is useful. These results can be later supported by future research in vivo.

**1.4 Significance of the Study**

This study will help lessen diabetic cases and the complications it creates. Diabetes can harm to people because it affects the different parts of the body. Having high sugar level might lead to amputations and even loss of lives. Nowadays several researches are found and discovered to regulate blood sugar. But most of these treatments are very expensive. This study is aimed to find the efficacy of fenugreek, betel leaves, cinnamon and jack fruit in regulating blood sugar. This study is very important because there is a high percent chance of producing new drugs or supplements using natural ingredients which will reduce blood sugar level.

**Chapter 2: Review of Literature**

**Starch**

Starch is the main carbohydrate storage in many plants. Starch from all plant sources occurs in the form of water soluble granules which differ in size and physical characteristics from species to species (Madihah et al., 2001). Starch is one type of complex sugar which is polysaccharide. Starch polysaccharides are macro molecules that consist of a large number of glucose units. They are sometime known as glycans. Starch is a mixture of two polysaccharides that built from glucose units which are amylose (a linear chain molecule) and amylopectin (a branched polymer molecule of glucose) (Sun et al., 2006; Madihah et al., 2001; Nigam and Singh,1995).

**Glucose**

Glucose (C6H12O6) contains six carbon atoms, one of which is part of an aldehyde group, and therefore known as an aldohexose (Figure 1). Glucose commonly presents in a form of white substance or as a solid crystal. Glucose also known as confectioners’ syrup and can be dissolved in water as an aqueous solution (Vandamme et al., 2002). The molar mass and density of glucose is 180, 16 g/mol and 1.54 g/cm3, respectively. The melting point of \_-D-glucose and \_-D-glucose is 146°C and 150°C, respectively.



Figure 1: Glucose structure (Vandamme et al., 2002)

**Enzymes**

Enzymes are specialized proteins that have a unique shape and chemical composition that creates a site, called an active site, for connection between the enzyme and other molecules called substrates. The shape and chemical makeup of the active site provides an area for part of the substrate to connect with the enzyme. Part of the active site holds the substrate and part catalyzes the reaction. The active site binds the substrate based on many chemical factors including shape, stereochemistry, electrical charge, and hydrophilic/hydrophobic considerations. The enzyme and substrate fit together through an induced fit, where the active site must have complimentary structures to those of the substrate to allow binding. The substrate is held in a position that is energetically favorable for the reaction to take place. The enzyme aids the process by allowing the reaction to occur at a faster rate and with lower energy requirements than under non-enzyme conditions. In this way, enzymes act as catalysts for biochemical reactions. Each enzyme is capable of catalyzing reactions with many successive substrate molecules without itself being consumed during the reactions.

The rate at which enzyme catalyzed reactions occur are often dependent on factors such as the pH of the solution, temperature of the solution, and concentration of the substrate.

The activity of enzymes can be inhibited. Studies of the methods by which enzymes are inhibited have practical applications. For example, many clinical therapies and biochemical research tools are based on enzyme inhibition. Enzyme inhibition is an extremely important area of research in the medical field. Lead, mercury, other heavy metals and nerve gases are extremely poisonous to humans because they are inhibitory to enzymes. For example, Pb++ can easily react with the sulphydryl (-SH) groups in a protein. Ag+ ions attach to the histidineside chains of invertase molecule and render it inactive.

**Herbs and Spices**

The use of synthetic chemicals as antimicrobials has greatly contributed to management of diseases, but indiscriminate application of chemicals has led to a number of ecological and medical problems due to residual toxicity, carcinogenicity, teratogenicity, hormonal imbalance etc. (Pandey, 2003). Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. Their medicinal use has been gradually increasing in developed countries. So, natural substances that can prevent AFB1 to toxicity would be helpful to human and animal health with minimal cost in foods and feed. Traditional [**medicinal plant**](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=medicinal+plant)**s** were applied by some researchers for their antifungal, antiaflatoxigenic and [**antioxidant activity**](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=antioxidant+activity) (Joseph *et al*., 2005; Kumar *et al*., 2007).

Ginger rhizome (*Zingiber officinale*), commonly known as ginger, is utilized worldwide as a spice and a flavoring agent. It has traditionally been used as a treatment for rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Afzal *et al*., 2001). Ginger extracts have been reported to have antioxidant (Masuda *et al*., 2004; Ajith *et al*., 2007a), anticancer (Lee *et al*., 2008), anti-inflammatory (Lantza *et al*., 2007) and antithrombotic effects (Thomson *et al*., 2002).

Cinnamon (*Cinnamomum eylanicum*) is a widely used spice and has many applications in perfumery, flavoring and pharmaceutical industries. Essential oils of cinnamon have been reported to have antimicrobial and antioxidant potency (RodrÂ´iguez *et al*., 2007; Singh *et al*., 2007). It has beneficial effects in the treatment of diabetes as it has insulin potentiating actions (Anderson *et al*., 2004).

Fenugreek (*Trigonella foenum graecum*) seeds are commonly used as spice in Indian homes. Fenugreek is a traditional medicinal herb which possesses antidiabetic and antiulcer potential (Sharma, 1986; Pandian *et al*., 2002). It prevents hyperlipidemia, atherosclerosis (Sharma *et al*., 1996) and cancer (Sur *et al*., 2001) in experimental animals. The seeds are reported to be rich in polyphenolic flavonoids (100 mg g-1) (Gupta and Nair, 1999) which has [**antioxidant activity**](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=antioxidant+activity) and was able to scavenge O2 and H2O2 (Kaviarasan *et al*., 2008).

Saponins in fenugreek may decrease glucose absorption in the digestive tract, and may be partially responsible for the herb's effect of reducing blood sugar. The trigonelline, nicotinic acid and coumarin components also have hypoglycemic activity. Trigonelline provides nicotinic acid, or niacin, during the seed roasting process. The amino acid 4-hydroxyisoleucine has been shown to increased use in secretion in studies with animals, according to the MSKCC.

Galactomannan fiber is another fenugreek component that may decrease glucose absorption from the gastrointestinal tract. A study published in the April–June 2005 issue of the South Asian Journal of Preventive Cardiology noted that including fiber in the diet improves glucose tolerance in diabetic patients. These foods decrease the rise in blood sugar after meals and also decrease the amount of medication required. Research noted in this study indicates that the gum type of fiber in fenugreek seed is most effective in lowering blood sugar.

The galactomannan rich soluble fiber fraction of fenugreek may be responsible for the antidiabetic activity of the seeds. Insulinotrophic and antidiabetic properties also have been associated with the amino acid 4-hydroxyisoleucine that occurs in fenugreek at a concentration of about 0.55%. In vitro studies have indicated that this amino acid causes direct pancreatic β-cell stimulation. Delayed gastric emptying and inhibition of glucose transport also have been postulated as possible mechanisms.

**Chapter 3: Experimental Design**

Hypothesis: Natural plant based inhibitors (Cinnamon, Fenugreek, Betel leaves, and Jackfruit), can inhibit the activity of the enzymes α-amylase & amyloglucosidase similar to the antidiabetic drug Acarbose because these reagents have flavonols or phenolic acids, and can cause Diet Induced Thermogenesis (DIT).

Independent Variable: Type of Plant Extracts tested for enzyme inhibition

Dependent Variable: The amount of glucose produced in the substrate, inhibitor enzyme reaction.

Constants:

* Amount of enzymes alpha amylase and amyloglucosidase used (1 mL)
* Time for which the reaction was measured (30 sec)
* Amount of starch used as a substrate (3 mL)
* Glucose measuring strips (Bayer Diastix)
* Amount of plant extracts used (2 mL)

Control: The FDA approved antidiabetic drug ‘Acarbose’ which inhibits enzyme activity and lowers glucose level.

Materials:

* Alpha Amylase - Fungal Amylase (100 grams) and Amyloglucosidase Enzyme (100 ml) from Carolina Biological Supply Company.
* Corn Starch (185 grams),
* Acarbose (10 tabs of 100 mg each),
* Trigonella foenum-graecum (Fenugreek leaves) - 200 grams,
* Artocarpus heterophyllus (Jackfruit) - 500 grams from Indian Grocery store,
* Cinnamomum zeylanicum bark - 100 grams,
* Trigonella foenum-graecum (Fenugreek seeds) - 500 grams,
* Piper betel (Betel) leaves - 10,
* Glucose (10, 1 gram tablets),
* 100 Diastix Glucose Test strips.

Lab Equipment: Electronic weigh balance, Electric Blender to powder agents, 50 Pipets. 25 stirrers, 10 plastic spoons, 25 test tubes capable of holding 100 mL of liquid, 10 Latex gloves, Lab notebook and pencil, 1 4GS iPhone (used as a timer) accurate to a tenth of a second, Distilled water, 3.78L, Strainer to filter the extract.

Experimental Procedure:

1. Gather all materials.
2. **Preparation of different Plant Extract**
	1. Use a digital balance to measure the quantity of reagents as described in appendix.

|  |  |  |  |
| --- | --- | --- | --- |
|   | **Quantity** **(in grams)** | **Water** **(in ml)** | **Final** **Concentration**  |
|  Alpha Amylase  | 5 | 100 | 50 mg/mL |
|  Amyloglucosidase | 1 | 10 | 100 mg/mL |
|  Starch | 20 | 100 | 200 mg/mL |
|  Cinnamon Powder | 5 | 100 | 50 mg/mL |
|  Fenugreek (Leaves) | 10 | 100 | 100 mg/mL |
|  Fenugreek (Seeds) | 10 | 100 | 100 mg/mL |
|  Jack Fruit | 100 | 100 | 1000 mg/mL |
|  Betel Leaves | 10 | 100 | 100 mg/mL |
|  Acarbose | 0.1 | 100 | 10 mg/mL |

* 1. Wash plant leaveswith distilled water and shade dry at room temperature.
	2. Powder the dried leaves using electric blender and stir the powder in water.
	3. Strain the mixture to prepare the required aqueous plant extract.
1. Create a **negative control** by dipping a test strip into a cup of **starch solution**. Wait 30 seconds, and observe the test strip. There should be no color change.
2. Create a **positive control** by dipping a test strip into a **glucose stock solution**. Make glucose solution by adding a 1 gram glucose pill to 100 mL of water. **Use this to create a standard curve for glucose.**
3. Add 3 mL of starch in each of the 5 test tubes using pipette.
4. Add 2 mL of Fenugreek leave extract into test tube1. Then add 1 mL of the prepared enzyme alpha-amylase having 100 mg/mL concentration.
5. Give 1 min for the reaction to occur. Dip the **Diastix test strip** into the mixture. Wait for 30 seconds before observing each test strip.
6. **Compare the color of the strip to the scale on the package**. Write down how much glucose is produced in this reaction in the data table.
7. In the remaining 4 test tubes **add fenugreek seed, betel, jackfruit, and cinnamon extract** and follow steps 5-7 to record glucose readings. This is a **semi-quantitative measure of glucose produced**.
8. Test each of the 5 plant extracts using the second enzyme amyloglucosidase following the steps 4 thru 8 using glucose test strips. Wait thirty seconds before observing each test strip.
9. Take 5 trials with the 2 enzymes to get enough sample data.
10. The Diastix strips have the enzymes, **glucose oxidase and peroxidase**, immobilized under a cellulose membrane at the tip. The glucose is permeable and in its presence, potassium iodide (used as a chromagen) is oxidized to brown iodine.

**Chapter 4: Data Tables**

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| 1. **The Amount of Glucose produced with Alpha-Amylase & different inhibitors**
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|  |  |  |  |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Type of Inhibitor tested** | **Glucose Produced (in mg/dL)** |  | **Average Glucose Produced (mg/dL)** | **Standard Error of the Mean** |
| **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** |
| **Water** | 1000 | 1000 | 1500 | 1000 | 1000 | **1100.00** | **129.0994** |
| **Acarbose** | 500 | 750 | 500 | 250 | 500 | **500.00** | **102.0621** |
| **Fenugreek Leaves** | 500 | 250 | 500 | 250 | 250 | **350.00** | **79.0569** |
| **Fenugreek seeds** | 500 | 500 | 500 | 500 | 250 | **450.00** | **64.5497** |
| **Jack Fruit** | 250 | 250 | 500 | 250 | 250 | **300.00** | **64.5497** |
| **Cinnamon powder** | 1000 | 750 | 1000 | 750 | 1000 | **900.00** | **79.0569** |
| **Betel Leaves** | 250 | 100 | 250 | 100 | 250 | **190.00** | **47.4342** |

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| Reaction with Starch + Inhibitor+ Enzyme (3 ml+ 2 ml + 1ml). |
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| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |

1. **The Amount of Glucose produced with
Amyloglucosidase & different inhibitors**

|  |  |  |  |
| --- | --- | --- | --- |
| **Type of Inhibitor tested** | **Amount of Glucose Produced in the reaction (mg/dL)** | **Average Glucose Produced (mg/dL)** | **Standard Error of the Mean** |
| **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** |
| **Water** | 2000 | 2000 | 1500 | 2000 | 1500 | **1800.00** | **129.0994** |
| **Acarbose** | 500 | 1000 | 500 | 750 | 500 | **650.00** | **129.0994** |
| **Fenugreek Leaves** | 500 | 750 | 500 | 750 | 500 | **600.00** | **79.0569** |
| **Fenugreek seeds** | 500 | 1000 | 750 | 1000 | 500 | **750.00** | **144.3376** |
| **Jack Fruit** | 500 | 1000 | 500 | 1000 | 500 | **700.00** | **158.1139** |
| **Cinnamon powder** | 1000 | 1500 | 1500 | 1000 | 1000 | **1200.00** | **158.1139** |
| **Betel Leaves** | 500 | 250 | 500 | 250 | 500 | **400.00** | **79.0569** |

Reaction with Starch + Inhibitor+ Enzyme (3 ml+ 2 ml + 1ml).

**Chapter 5: Data Graphs**

**The amount of glucose produced from starch in the presence of different inhibitors**

**Chapter 4: Results and Discussion**

**Results and Conclusion:**

The results indicate that extract of Piper Betel showed maximum alpha-amylase and amyloglucosidase inhibitory activity. Jackfruit and fenugreek showed appreciable inhibition activity. When the inhibitors were mixed with starch as substrate, SI + E, competitive inhibition was observed for both the enzymes tested. The standard error of mean was about 5% so the hypothesis that the natural products can be used to regulate blood glucose can be accepted. The **results support the hypothesis** that the flavonoids and polyphenols present in these medicinal plant extracts can lower blood glucose levels. They are potential candidates for diabetes management and warrants further preclinical evaluation as hypoglycemic drug.

The present study used glucose strips to semi-quantitatively measure the effect of various inhibitors using the standard curve created with glucose solutions. The Diastix strips are not very sensitive to smaller changes in the glucose concentration. **The colors can be subject to individual reading errors**. For quantitative measurement of glucose quantity, an attempt was made to use spectrophotometer. But the color of the natural products interfered with the absorbance and precise inference could not be made. If the **plant extracts could be prepared using ethanol and centrifuge**, color change could be measured accurately. When measuring the enzyme inhibition, each of the **natural extract had different consistency** which could have affected the amount of glucose produced in the enzyme reaction.

In the future two natural products could be mixed together (fenugreek + Betel leaves) to see if the effect of the mixture is any different than that of the natural inhibitors themselves. This study can be done in a commercial lab using high precision instruments and spectrophotometer to check for absorbance of product formed at different wavelengths.

**Discussion and Future Research**

The experimental results will **stimulate further research** on pharmacologically active phyto constituents obtained from medicinal plants. Each year millions of dollars are spent on diabetes treatments which have some limitations, significant side effects. Acarbose delays glucose absorption and causes gastrointestinal side effects like diarrhea, flatulence. This experiment can be repeated at the body temperature (37 °C) to check if the same inhibition is recorded and also to do a time course to check if there is difference in the rate of reaction.

When a large number of compounds are to be tested, or when compounds are synthesized with minor modifications in functional groups or different percentages of extract, **in vitro** data is useful. These results can be later supported by future research in vivo.

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