Evaluation of The Hypoglycemic Effects of Dried Leaves of *Stevia rebaudiana* (Bertoni), on The Treatment of Type-II Diabetic Patients of Bangladesh.

A Dissertation Submitted to the Department of Pharmacy, East West University in Partial Fulfillment of the Requirement for the Degree of Masters of Pharmacy.

Supervised by

Dr. Chowdhury Faiz Hossain
Professor, Department of Pharmacy
Dean, Faculty of Science and Engineering
East West University.

Submitted by

Rezwana Razzaque Naomi
ID: 2010-3-79-024

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EAST WEST UNIVERSITY

Declaration by the Research Candidate

I, Rezwana Razzaque Naomi declare that the dissertation entitled "**Evaluation of the hypoglycemic effects of Dried Leaves of *Stevia rebaudiana* (Bertoni), on the treatment for type-II diabetic patients of Bangladesh" ,** submitted by me to the Department of Pharmacy, East West University , in the Partial fulfillment of the requirement for the award of the Degree of Masters of Pharmacy, is a record of original research work carried out by me during 2011-2012 under the supervision and guidance of **Dr. Chowdhury Faiz Hossain**, Professor, Dept. of Pharmacy, East West University and it has not formed the basis for the award of any other Degree/ Diploma/ Fellowship or other similar title to any candidate of any University.

Dhaka-1212 Signature of the candidate

Date: 31.05.12 (Rezwana Naomi)

This Theses paper is Dedicated to My Family

Thesis Certificate

This is to certify that entitled "**Evaluation of the hypoglycemic effects of Dried Leaves of *Stevia rebaudiana* (Bertoni), on the treatment for type-II diabetic patients of Bangladesh" ,** submitted to the Department of Pharmacy, East West University, in the Partial fulfillment of the requirement for the award of the Degree of Masters of Pharmacy, is a record of original research work carried out by Rezwana Razzaque Naomi during the period 2011-2012 of her research in the Department of Pharmacy, East West University, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree/Diploma/Fellowship or other similar title to any candidate of any University.

Dhaka-1212

Date:31.06.2012

(**Dr. Chowdhury Faiz Hossain)**

Principle Supervisor

Professor, Department of Pharmacy
East West University.

I endorse the above statements:

(Dr. Sufia Islam)
Chairperson
Department of Pharmacy
East West University.

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Abstract

Stevia rebaudiana Bretoni, a native plant of Paraguay and Brazil, known for its sweetener and antidiabetic property world wide. However, the mechanisms for the blood glucose-lowering effect remain unknown. Stevioside, the main glycoside (250-300 times sweeter than sugar) present in the leaves of *Stevia rebaudiana*, is varies 4-20% depending on the soil property, cultivation technique, weather. Recently the plant *Stevia rebaudiana* is cultivated in Bangladesh. But there is no clinical report on the antidiabetic property of this plant in Bangladeshi population.

The study was conducted to investigate the effects of powdered form of stevia leaves on blood glucose concentration of randomly selected 20 type-II diabetic patients of Bangladesh. Powdered form of Stevia leaves was given by oral administration at 2 gm/day in two divided doses, half teaspoon, 1gm, in morning and half teaspoon, 1gm, in evening). Changes in the blood glucose levels were measured by glucometer and the obtained data was analysis by using one sample t-test.

Mean fasting blood Glucose level of those patients before the use of Stevia powder was found to be 13.74±0.76 mmole/L. The administration of 2 gm/ day of powdered leaves of stevia leaves in those patients showed statistically significant reduction of blood glucose level in 8.59±0.50 mmol/l with in 6 weeks. The patients were monitored up to 12 weeks and the reduction level of fasting blood glucose was maintained.

Similarly, mean blood Glucose level of those selected patients two hours after breakfast before the use of Stevia powder was found to be 14.98±0.81 mmole/L. The administration of powdered Stevia leaves in those 20 patients showed statistically significant reduction of blood glucose level after two hours of breakfast was 9.58±0.42 mmol/l within 6 weeks. The patients were monitored up to 12 weeks and the reduction level of fast blood glucose was maintained.

Thus, the powdered Stevia leaves produced significant hypoglycemic effects on blood glucose concentration when compare with the blood glucose level of before using stevia green powder of 20 type-II diabetic patients. From this study, it was observed that the compound *Stevia reabaudiana* green leaf powder may have a potential role as antihyperglycemic agents in the treatment of type-II diabetes mellitus in Bangladeshi population.

Key words: *Stevia rebaudiana*, Stevioside, Diabetes mellitus.

Introduction

1.1. Introduction:

The rapidly increasing Diabetes mellitus is becoming a serious threat to mankind health in all part of the world. The control and treatment of diabetics and its complication mainly depend on the chemical or biochemical agents, but the fact is that it has never been reported that someone had recovery totally from diabetes. Diabetes mellitus is a major health problem not only in urban but also in the rural areas of Bangladesh. This systemic illness occurs as a result of relative or absolute deficiency of insulin action on blood sugar. Although insulin has been used successfully in insulin-dependent diabetes mellitus but a suitable drug is yet to be available which can cure the disease permanently.

Even insulin can not be given orally and daily intake through injection is obviously troublesome and insulin resistance is another drawback for patients taking it for a long period of time. On the other hand, oral hypoglycemic agents such as Glimepiride, glibenclamide etc. have some adverse effects and these are unavailable in rural area also. Now a day, various medicinal plants are becoming very popular for the treatment of different diseases in our country as well as all over the world. Ethno botanical studies of traditional herbal remedies used for diabetes around the world have identified more than 1,200 species of plants with hypoglycemic activity[1]. *Stevia rebaudiana* Bertoni is one of them which are herbaceous perennial plant native to subtropical and tropical rainforest areas of South America (Brazil, Venezuela, Colombia and Paraguay). The leaves are used traditionally in various regions of the world including China, Japan, Korea, Taiwan, Thailand, Malaysia and Paraguay. The leaves have been known to contain 100 useful alkaloids among other pharmacologically active compounds. It has been used for the treatment of diabetes and its anti-diabetic effect has been evaluated in diabetic animals in many countries and significant hypoglycemic activities of powdered form of Stevia (*Stevia rebaudiana* Bertoni) leaves have been reported. However, its effects have not yet been investigated in Bangladesh. Therefore, the present research work was undertaken to study the hypoglycemic effect powdered form of Stevia leaves on treatment for the diabetic patients.

1.2. Diabetic patients in Bangladesh to hit 1.11 cores by 2030:[2]

Diabetes is one of the most prevalent and devastating chronic non-communicable diseases having serious health, economic and social consequences[2].An emerging epidemic of diabetes is affecting more people in low income than high income countries. It is estimated that the number of people with diabetes globally will increase from current 171 million to 366 by 2030[3.] Diabetes and its complications place a more serious burden on individuals and families in low income countries where access to adequate treatment is poor and subsidy for treatment is generally unavailable. An ageing population, rapid urbanization with an associated more sedentary lifestyle, an altered diet high in energy dense processed foods which replaced traditional healthy diet consisting of plenty of starchy foods such as wholegrain bread, rice, some protein-rich foods and plenty of fruit and vegetables, and increased obesity are contributing to emergence of diabetes in several economies[4]. People in poor settings are often exposed to undernutriton during prenatal life and born with low birth weight[5.] Smaller size at birth has been shown in several studies to be associated with, higher fasting glucose concentration, impaired glucose tolerance, and development of type 2 diabetes in adult life[6.] A high prevalence of low birth weight in low income countries might be one of the major determinants of the high prevalence of glucose metabolic abnormalities. Studies in various populations in Bangladesh have reported a prevalence of diabetes from 4 % to 13 % among adults with some variations by urban and rural settings. Wild *et al* estimates that Bangladesh currently has over three million people with diabetes and this number will reach 11 million by the year 2030 [3.]

While diabetes is considered a serious disease, once diagnosed healthy lifestyle and adequate clinical care can improve outcome. Prediabetic conditions (impaired fasting glucose [IFG] or impaired glucose tolerance [IGT]) are rarely recognized but they carry the same cardiovascular risk as diabetes[7]. The population with prediabetic conditions is always much larger than the diabetic population, but offers an opportunity for primary prevention. Studies in Finland, the United States, and China demonstrated that diabetes can be prevented in more than half of the individuals with prediabetic conditions through interventions to modify lifestyles[8-10.]

1.3. Responses to "Type 2 Diabetes Treatment with Herbal Remedies"

The great majority of diabetes sufferers are affected by type 2 diabetes. This usually occurs in adulthood and it is also known as non-insulin dependent diabetes. People who are overweight, who have a sedentary lifestyle or who have had diabetes in the family are running high chances of developing the disease. Type 2 diabetes evolves progressively, leading to serious complication in time. If ignored or mistreated, diabetes can cause heart disease, kidney failure, blindness and amputation of limbs. It all starts with symptoms like frequent urination, increased hunger, increased thirst, blurred vision, constant fatigue and slow healing of wounds. A physician needs to be consulted right away because under no circumstances should diabetes be left untreated. Herbal treatments can also help the sufferers and they can be an adjuvant of the traditional treatment scheme.

Herbal remedies for type 2 diabetes include either plants that are capable of balancing the blood sugar or plants that prevent or eliminate diabetes complications. With the appropriate herbal treatment you can live a long and happy life without ever suffering of the terrible complications that usually accompany diabetes.

Leaves from stevia plants offer several important benefits to people with diabetes and metabolic syndrome. The plant's honey-like flavor makes it a viable and tasty alternative to glucose-spiking sweeteners like sugar and corn syrup. Additionally, people with type II diabetes may benefit from stevia's ability to enhance insulin production and insulin sensitivity; this, in turn, may help stabilize blood glucose levels. While evidence is limited, some nutritionists also suggest stevia as a low glycemic index sweetener for people with reactive hypoglycemia, or pre-diabetes. Experts have hypothesized that it may prevent the blood sugar fluctuations that lead to the development of type II diabetes.

1.4. Stevia in Bangladesh:

Stevia research is in progress in the food biochemistry laboratory of Biochemistry Department, Bangladesh Agricultural University under the leadership of Prof. Dr. M. Afzal Hossain in collaboration with Bangladesh Sugar Research Institute (BSRI), Iswardi from the very beginning of the introduction of this plant in Bangladesh from Bangkok.

However, no work has been carried out so far to utilize stevia extracts in the formulated food for diabetic subjects. Based on its potentiality as a food sweeter Prof. Dr. M. Afzal Hossain with his group has a vision to use stevioside extracts initially in milk products, biscuits, bread, chocolates, ice-creams, and soft drinks in order to meet the demand of sweet for diabetic subjects.

At the moment in his laboratory a group of post-graduate students are involved in a research project entitled " Formulation of cereal & legume based therapeutics food containing semi purified antidiabetic and cholesterol lowering active principles from stevia plant"

Recently, a team of post-graduate students from the same Department visited NABISCO biscuit factory to demonstrate the use of stevia extract (extract produced in the BAU-Food Biochemistry lab) as an alternative to table sugar as an ingredient of biscuit. It is very encouraging to note that the sugar free biscuits produced were sweet and generally acceptable. Research work is in progress to improve the color, texture and delicacy of the biscuits. For the consumers' acceptance it is imperative that in addition to dietary quality, the product(s) is also attractive.

1.5. Objective:

Most of us love sweetmeats. Our palates lust for ice cream, our mouths water at the thought of varieties of sweetmeats, our parched throats yearn for soft drinks, while visions of sugar plums dance in our heads. To satisfy these craving, we consume about one hundred pounds (45 kg) of sugar per person per year. That's about 120 grams per day. We're predisposed to seek out sugar when we can find it. After all, sugar (sucrose) is a carbohydrate. Sugar is metabolized directly into blood sugar or glucose, which fuels our brain and muscles. The purer the source, the faster it gets into the bloodstream, bypassing much of the digestive process.

Eating sugar shoots our blood sugar levels up and triggers a spike in the hormone insulin, which is needed to prep our cells to absorb the sugar. If there are no other nutrients to sustain our blood sugar, it crashes as quickly as it rises - and we crave another hit. This is how sugar addiction begins. Moreover, sugar floods us with pleasure by stimulating the release of the

neurotransmitter serotonin, and probably other mood-elevating substances. Scientists report that eating chocolate initiates a brain response similar to falling in love.

Although we get quick calorie from sucrose, it has several adverse health effects. The most common is tooth decay, in which bacteria in the mouth turn sucrose into acid that attacks tooth enamel. Sucrose has high calorie content and is also believed to cause obesity and is not ideal for our waistline. People with diabetes need to control their intake of sucrose. There have even been some controversial suggestions that excessive sugar consumption may play a role in certain degenerative diseases. It is commonly believes that eating too much sugar will cause some children to become hyperactive.

The four primary compounds widely used as sugar substitutes in the United States and many other countries are saccharin, aspartame, sucralose, and cyclamate. However, there is ongoing controversy over the supposed health risks of the artificial sweeteners such as saccharin and aspartame. Some studies have shown that they cause brain tumors as well as lymphatic cancers in laboratory animals. Multiple scientific studies have demonstrated health risks of saccharin to humans. For various adverse effects of artificial sweeteners and sugar (sucrose), the search for natural plant sweeteners has been intensified in recent years. Meanwhile, several compounds are discovered and some of them being developed for commercial application.

One of the best known natural sweet compounds is stevioside, a diterpenoid glycoside which occurs in the leaves of a plant named Stevia rebaudiana belonging to the Composite family. It is a remarkable South American plant that has become widely used in certain parts of the world as a natural sweetening agent and dietary supplement. Purified extracts of Stevia have been used as sweeteners and flavor enhancers in the food industry in Japan for over a quarter of a century, and have been found to be up to 300 times sweeter than sucrose. So a small portion of Stevia will sweeten even a strong cup of tea. Stevia has been used in many other countries for over 400 years without any side-effect.

The advantages of sweet stevia leaves and stevioside are manifold. These are: non-toxic, non-calorific, heat stable, not fermentative, flavor enhancing, 100 % natural, no aftertaste or bitterness, non-carcinogenic, non-addictive sweetener for children, and an intense sweetener compared to sucrose. Due to non-calorific property, it is absolutely safe for diabetics,

phenylketonurie (PKU) patients and slimming people. Stevia can be used for anything you might use sugar in, including baking. For men and women who want to move through their cravings for sugar without artificial chemicals, Stevia is a great option.

Stevia is recently introduced in Bangladesh; however, it may take time to acclimatize this plant for commercial use as a natural sweetener. As the content of stevioside in the leaves of Stevia is largely varies between 4.0 % and 20 % of the dry weight of the leaves depending on the cultivar and on growth conditions, extensive research activities are needed to quantify stevioside content in leaves and find out suitable cultivation techniques for commercial production of stevia in different soils of Bangladesh.

Stevia *(Stevia rebaudiana*) is an exotic plant in our country. Stevia was first introduced in Bangladesh from Thailand by Bangladesh sugarcane Research Institute (BSRI).BRAC, Tissue Culture Lab, Department of Botany R.U, and Powerade-Agro–hi-tech Limited have developed method for *in vitro* production of stevia Seedling and small scale cultivation and marketing of stevia dried leaves, but no report on the clinical test and nutritional aspects of this new crop were introduced in Bangladesh. As the sweet stevioside content in its leaves varies from 4-20%depending on the cultivar, soil, water and other environmental factors it is now essential to develop on appropriate cultivation technique and to determine the chemical and bio-chemical constituent and clinical test for its large scale production in Bangladesh. The fore sent piece of work was therefore undertaken with the following objectives:

- To examine the stevioside (%) content in dried leaves of stevia.

- To evaluate the hypoglycemic effect of dried leaves of stevia for the treatment of diabetic patients in Bangladesh.

The diabetic patients and any healthy sweet lovers may satisfy their emotional incentives by natural no calorie sweeteners such as stevia or stevioside as the safe alternatives of sugar. As stevia is introduced in Bangladesh, research efforts are needed in developing appropriate techniques for commercial cultivation and utilization of this magic plant as a safe alternative of sucrose.

2. About the Plant:

Figure 1: ***Stevia rebaudiana* Plant**

Scientific classification:

Kingdom:	Plantae
Order:	Asterales
Family:	Asteraceae
Tribe:	Eupatorieae
Genus:	***Stevia***
Species:	***Stevia rebaudiana***

2.1. About the plant:

Stevia is a genus of about 240 species of herbs and shrubs in the sunflower family (Asteraceae), native to subtropical and tropical regions from western North America to South America. The species *Stevia rebaudiana*, commonly known as **sweetleaf**, **sweet leaf**, **sugarleaf**, or simply **stevia**, is widely grown for its sweet leaves.

Stevia plant was first studied by a Botanist from Paraguay named Antonio Bertoni in 1887.He wrote the first research articles about stevia at the beginning of the 20th century. The molecules that cause the sweetness in the stevia leaf were discovered and documented in 1931. Eight new photo chemicals called glycosides were identified, Stevioside was found to be the sweetest of all eight glycosides. The steviol glycosides are more sweet taste in the leaves of the stevia plant *(Stevia rebaudiana* Bertoni). These are 40 to 300 times more sweater than sucrose. They are heat-stable, pH-stable, and do not ferment. They also do not induce a glycemic response when ingested, making them attractive as natural sweeteners to diabetics and others on carbohydrate-controlled diets.

Steviosides are used for preparation of pharmaceutical products (helpful for hypoglycemia and diabetes), for flavor enhancer, in food and beverage application as a replacement for sugar and artificial sweeteners, in herbal tea etc.

Stevia (Stevia rebaudiana) is an exotic plant in our country. It has both economical and medicinal importance. Stevia (also called sweet leaf or sugar leaf) is a genus of about 150 species of herbs and shrubs in the sunflower family (Asteraceae), native to subtropical and tropical South America and Central America. Stevioside is a glycoside of the diterpene derivative of steviol. Steviol glycosides are natural constituents of the plant Stevia rebaudiana Bertoni.

It grown as a perennial in subtropical and mild temperate regions but must be grown as an annual in mid and high altitude. Average maximum and minimum temperature between 10 ^{0}C- 37 ^{0}C during the active crop growth period. Relative humidity ranging from 65 – 85 %. Stevia can tolerate rains but is prone to frost. Thrives best in well drained red soil and sandy loam soil with a pH ranging from 5.5- 7.7.

Propagation through stem cuttings as well as through seeds. Transplanted rooted cuttings give better results. Rooted cuttings of 4- 5 weeks old. These are transplanted in the field during March- April and June- July at spacing of 45 cm x 45 cm.

Man does not produce stevioside, or synthesize it in a laboratory or manufacture it. Man simply *extracts* it, just like nature produced it from the tiny leaf of a beautiful little South American plant. Thus stevioside is not "artificial" or "synthetic"; it is truly a natural product

and also a "low-calorie" one. Stevia is likely to become a major source of commercial sweetener for the growing natural food market in the future. The task at hand is to adapt stevia from a wild plant to a modern crop well suited to efficient mechanized production. In Canada, the necessary steps taken are: development of seed, seedling and crop production system including information on optimized crop inputs; weed and disease control; harvest and handling methods and a breeding program aimed at optimizing glycoside content and sensory characteristics. At present understanding the biology of the stevia plants and biochemistry of the sweet glycosides are prerequisites for conversion of stevia to a modern crop.

Stevia has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives. Stevia also has shown promise in medical research for treating such conditions as obesity and high blood pressure. Stevia has desirable effect on blood glucose, even enhancing glucose tolerance, and therefore it is attractive as a natural sweetener to diabetics and others on carbohydrate-controlled diets. Stevia is widely used as a sweetener in Japan, and it is now available in the US and Canada as well.

The plant leaves, their aqueous extract and purified steviosides are used as sweeteners. Stevia sweeteners have been produced commercially in Japan since 1977 and are widely used in food products, soft drinks (including Coca Cola), and for table use. Japan currently consumes more stevia than any other country; it accounts for 40% of the sweetener market. Today, stevia is cultivated and used in food elsewhere in East Asia, including China (since 1984), Korea, Taiwan, Thailand, and Malaysia. It is also found in part of South America (Brazil, Paraguay, and Uruguay) and in Israel. China is the world's largest exporter of stevioside.

2.2.Folk medicine and research

For centuries, the Guaraní peoples of Paraguay used stevia, which they called **ka'a he'ê** ("sweet herb"), as a sweetener in *yerba mate* and medicinal teas for what they believed to treat heartburn and other ailments.[11] Current research has evaluated its effects on obesity and hypertension.[12,13] Stevia has a negligible effect on blood glucose, and may even enhance glucose tolerance; it may be useful as a natural sweetener for diabetics and others on carbohydrate-controlled diets.[14]

2.3. Stevia Leaves:

Fresh leaves: have a mild licorice flavor. This is the simplest form of Stevia in its most natural and unrefined state. The leaves are used to prepare sauces but are best in herbal teas and for direct consumption. They do not dissolve. In various markets they may be purchased loose or in tea bags. They are 15 to 30 times sweeter than sugar.

Figure 2: Fresh leaves of ***Stevia rebaudiana***

Dried leaves: are 10 to 15 times sweeter than sugar. To dry them, one just removes all the water (the easiest way is to dry them with a dehydrator, but drying them in an oven on the lowest setting will also work), which allows them to have an extended storage period. They have the same uses as fresh leaves but are also for industrial purposes, to extract the Stevioside.

Figure 3: Dried leaves of ***Stevia rebaudiana***

Powdered or ground leaves: They have a greenish, leaf color and are used as a flavor enhancer or sweetener in teas, salads, fruit, and coffee, among others. Ground Stevia leaves do not dissolve.

Figure 4: Powdered or ground leaves of ***Stevia rebaudiana***

2.4. Glycosides Present In Stevia:

The natural extract *of Stevia Rebaudiana* is mainly composed of sweet glycosides. These molecules are biosynthesized and stored by the stevia plant in the leaves and have been identified as stevioside, rebaudiosides and dulcosides. Other natural components found in stevia are vitamins, minerals, and nutrients such as: ascorbic acid, beta carotene, calcium, chromium, iron, magnesium, phosphorous, potassium, sodium, fluride, zinc, selenium, cobalt and proteins.

In terms of weight fraction, the four major steviol glycosides found in the stevia plant tissue are:

- 5–10% stevioside (250–300X of sugar)
- 2–4% rebaudioside A — most sweet (350–450times of sugar) and least bitter
- 1–2% rebaudioside C
- ½–1% dulcoside A. (see structure in literature review)

The predominant sweetener components of stevia extracts have been identified as stevioside and Reb A.

Stevioside is a glycoside of the diterpene derivative steviol (ent-I 3-hydroxykaur-I 6-en-19-oic acid). Steviol glycosides are natural constituents of the plant *Stevia rebaudiana Bertoni,* belonging to the Asteraceae family. The leaves of S. *rebaudiana Bertoni* contain eight different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting about 5-10% in dry leaves. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat.

Special feature of stevioside:

- 100% NATURAL
- No calories
- Control blood sugar levels, unlike sugarcane sugar does
- Heat stable to 198-200 Celsius
- Non-fermentable, Non discoloring
- Nature flavor enhancer
- Recommended for diabetics
- Non toxic readily soluble in water
- P^H stable
- Stable to drying, preservation and storing.
- Extensively tested in animals.
- Anti-bacterial effects.
- Stevia prevent tooth decay.

2.5. Stevia verses insulin & anti-diabetic medicine:

Type II diabetes is impossible to cure and needs vast amounts of medicinal treatment to control it. Not only is Type 2 diabetes a preventable condition, it can even been cured with the simplest herbal treatments which are readily available along with a proper diet.

Insulin's main role is not to "control" blood sugar. When blood sugar becomes elevated it is a signal for insulin to be released to direct the extra energy into storage. A small amount is stored as a starch called glycogen in our body, but the majority is stored as our main energy supply — fat. Thus, in this regard insulin's major role is not to lower sugar, but to take the extra energy and store it for future times of need. In other words insulin lowers glucose as a side effect of directing the extra energy into fat storage.

Type II diabetes is caused when the pancreas doesn't produce the proper amount of insulin, causing blood sugar levels to increase. Some believe that age, obesity, pregnancy, and certain illnesses give rise to this condition. This is not true at all POOR DIET causes Type 2 diabetes. While modern medicine uses synthetic treatments to cure the symptoms, they can actually contribute to worsening the overall health. Drugs like Avandia and Rezulin have been known to produce life-threatening side effects, which leave patients in poorer health than when they began taking them.

But drugs are not the only solution. In fact, more and more patients are beginning to realize the amazing healing powers of herbs, which have long since been used in countries around the world to treat, and cure, conditions like asthma, heart disease, blood pressure, cancer and diabetes.

In fact, the curative power of herbs is so strong that they are often included in medicines, an estimated 25 percent of drugs contain at least one plant-based extract. Plenty of exercise, proper diet and the use of the proper herbs can help to overcome Type II Diabetes and get back a healthy body. Controlling Type II diabetes and get back a healthy body. Controlling Type II diabetes with herbs and diet is not easy but the rewards are improved health.

Stevia is rapidly gaining ground as a zero-calorie plant-based sweetener. However, it is also recognized in traditional healing as a means to treat diabetes. The natural green Stevia leaf powder take will help modulate the blood sugar levels will have beneficial effects on glucose tolerance and therefore potentially help with diabetes. Stevia green powder are good for people that are controlling there diabetes with insulin or herbs. While research is still underway to confirm the effectiveness of Stevia in treating diabetes, its use as a sugar replacement cannot be questioned. Currently, the FDA has recognized Stevia extract for use in food products. When purchasing Stevia extract, ensure that it contains no fillers or additives and that it is Certified Organic.

2.6. Stevioside versus Artificial Sweetener:

Artificial sweeteners such as aspartame, saccharine, cyclamate etc. are used for diabetic subjects. But all of these artificial sweeteners have limitations e.g. aspartame a dipeptide of the amino acids aspartic acid and phenylalanine has been the subject of a vigorous public

controversy regarding its safety and the circumstances around its approval because it has negative effects on headaches, brain tumors, brain lesions, and lymphoma. Moreover there is also a positive correlation between saccharin consumption and increased frequency of cancer especially bladder cancer. Since cyclamates appear to affect cells involved in the production of spermatozoa, the question has also been raised as to whether they may also be capable of damaging male reproductive DNA.

The sugar or sucrose is the most popular sweetener in the world. However, for adverse health effects of sucrose and known artificial sweeteners, interest in and search for no calorie natural sweeteners has been intensified in recent years. Very fortunately, stevioside was found which can satisfy the urge for sweet consumption of diabetic subjects.

2.7. Political Controversy about Stevia:

The problem has been that stevia has not been used in making commercial products due to pressure from the sugar and artificial sweetener industries.

Some countries like USA, Singapore and Hong Kong have restricted the use of stevoside by on "toxicological information on stevia is inadequate to demonstrate its safety". In 1991, based on an anonymous complaint, the United States Food and Drug Administration (FDA) labeled stevia as an "unsafe food additive" and restricted its import. The reason was limited toxicological information on it. This ruling was controversial, as stevia proponents pointed out that this designation violated the FDA's own guidelines under which any natural substance used prior to 1958 with no reported adverse effects should be generally recognized as safe. Similarly, the FDA requires proof of safety before recognizing a food additive as safe and a similar burden of proof is required for the FDA to ban a substance or label it unsafe. Now the question arises that why some countries restricted the use of stevia? It is believed that they acted in response to lobby by sugar industry and the FDA action against stevia "a restraint of trade to benefit the artificial sweetener industry.

On the contrary, millions of Japanese people have been consuming stevia for over thirty years with no reported or known harmful effects. In 2006, the World Health Organization (WHO) performed a thorough evaluation of recent experimental studies of stevioside and steviols conducted on animals and humans, and concluded that "stevioside and rebaudioside A are not

genotoxic in vitro or in vivo .The US FDA itself states: "the stevioside, has reportedly been approved for use in foods in Brazil and Japan. The product is used in these countries as a table-top sweetener in virtually all food commodities and as a flavor enhancer in such products as teas."

2.8. Absorption, Metabolism and Excretion:

- Absorption studies have been performed in *in vitro* assays (everted intestines, Caco-2 cell layers) as well as in *in vivo* studies. From the different studies it is obvious that the absorption of steviol glycosides is extremely low, but the absorption of steviol, the aglycone of steviol glycosides, is good.

-Metabolism studies have been performed in different animals, in bacteria and in *in vitro* assays as well as in human volunteers. Whole animal studies using single and repeat doses were done.

None of the digestive enzymes from the gastro-intestinal tract of different animals and man are able to degrade stevioside into steviol, the aglycone of stevioside. Moreover, incubation of stevioside (97% purity) in 3 freshly taken samples (intubations) of stomach juice at 37°C for 6 h (1 mg/ml) revealed that there was no degradation of stevioside. This means that the stevioside ingested, reaches the intestines intact.

In human metabolism studies, no free steviol was found in the blood or urine, but steviol glucuronide was present as typical excretion product. Whereas Genus *et al*. did not find stevioside in blood or urine samples (below the detection limit of the UV detector used by use of LC-MS, found small amounts of stevioside in plasma of 7 volunteers (maximum concentration 0.1 µg/mL plasma) with a large inter-individual variation. In urine only very small traces of stevioside were detected in amounts that were too low being quantified. After enzymatic hydrolysis by β-glucuronidase/sulfatase, steviol could be set free out of the steviol glucuronide that was the only conjugate detected in urine.

No free steviol was detected in urine. After enzymatic hydrolysis of urine extracts by β-glucuronidase/sulfatase, steviol was found as the only aglycone present. There was no

indication for the occurrence of, e.g., steviol sulfates even after large scale extractions of urine. As no other metabolites were found, the following excretion route is suggested.

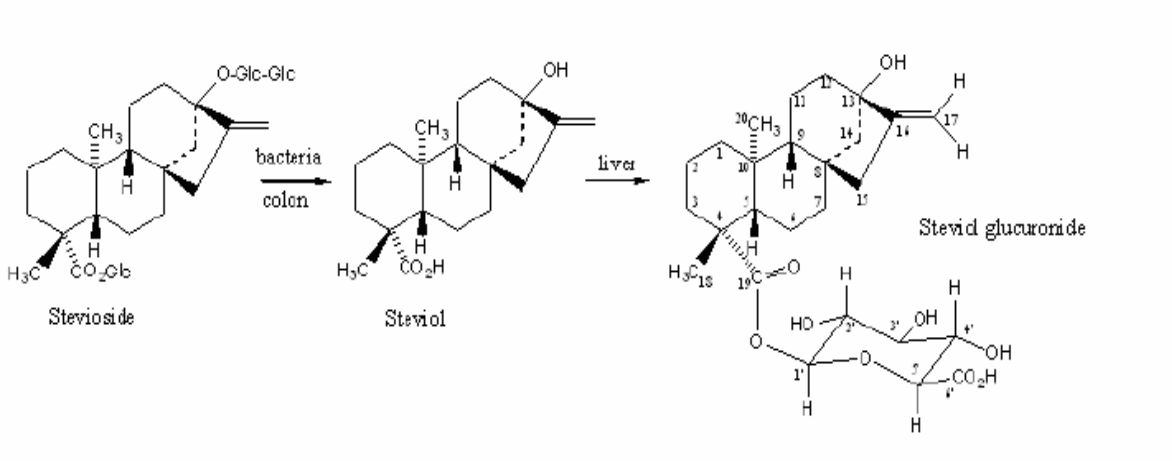


Figure 5: Hypothetical route from dietary stevioside to steviol glucuronide in human urine.

After degradation of stevioside to steviol by bacteria of the colon, part of the steviol is absorbed by the colon and transported to the liver by portal blood. In the liver, the steviol glucuronide is formed, which is released into the blood and filtered out by the kidneys into the urine. The high levels of steviol glucuronide in the urine suggest that there is no accumulation of steviol derivatives in the human body. The steviol glucuronide still present in the blood is expected to be excreted in the urine during the next 24 h. Besides steviol glucuronide, no free steviol or any other of the possible steviol metabolites could be detected in blood or urine. Hepatic metabolism of steviol is extremely low, if existing at all which is in agreement with results of Koyama et al. (2003b) who demonstrated in *in vitro* experiments that the steviol metabolism by human microsomes was 4 times lower than that of rat microsomes, and this last one was already very low.

2.9. Pharmacological effects

Some pharmacological effects of very high doses (750 to 1500 mg/day) were observed as decreased blood pressure in hypertension, and lowering of blood glucose in hyperglycemia. However, these high doses to provoke pharmacological effects will never been reached by the steviol glycosides used as a sweetener as only low amounts will be used, estimated to be 5-10 x lower than the amounts producing the pharmacological effect.

3. Literature review:

The leaves of Stevia, a perennial bush native to eastern Paraguay, have been recorded as a sweetener for many years. It has been largely ignored by most of the world until the last ten to fifteen years. However, there is now rapidly expanding interest in Stevia as a natural alternative to artificial sweeteners because it is not only a 'natural' calorie free product but also has other advantages over the currently used chemical artificial sweeteners, such as saccharine, aspartame and cyclamate.

3.1 Chemical Literature review:

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

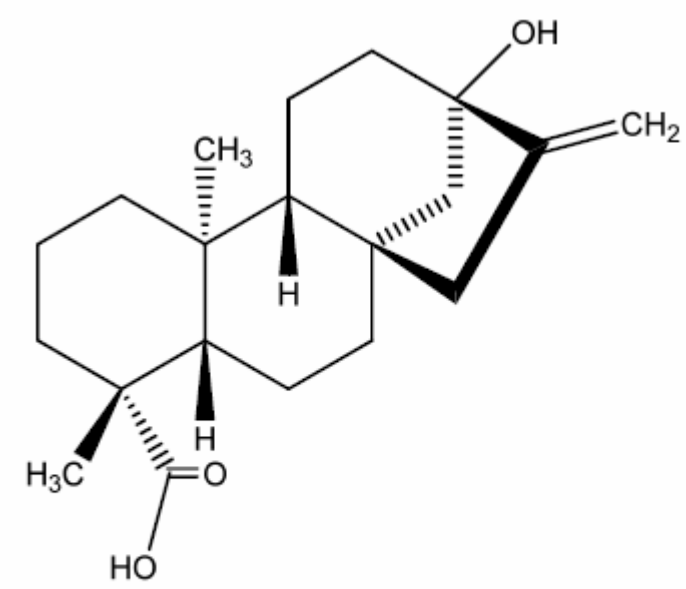
3.1.1. The Chemistry of the Diterpene Glycoside Sweeteners:

The sweet diterpene glycosides of stevia have been the subject of a number of reviews.[15, 16, and 17.] Although interest in the chemistry of the sweet principles dates from early in the century, significant progress towards chemical characterization was not made until 1931, with the isolation of stevioside[18] Treatment of this substance with the digestive juice of a snail yielded 3 mol of glucose and 1 mol of steviol, while acid hydrolysis gave isosteviol [19].Isosteviol was also obtained when steviol was heated in dilute sulfuric acid. Subsequent studies have led to the isolation of seven other sweet glycosides of steviol. Typical proportions, on a dry weight basis, for the four major glycosides found in the leaves of wild stevia plants is 0.3% dulcoside, 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside.

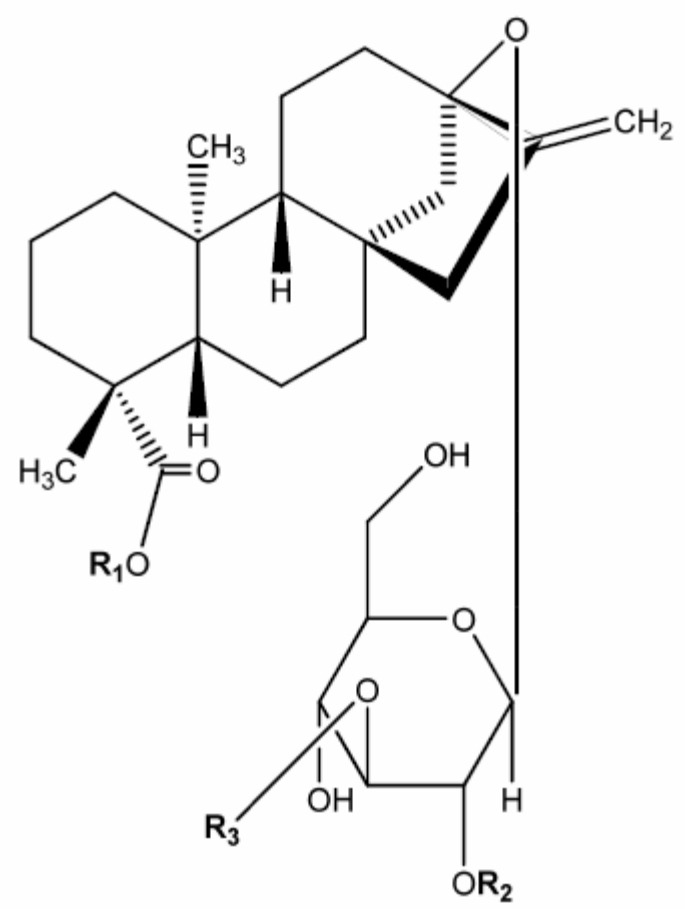
3.1.2. Structure of Steviol, Isosteviol and Stevioside:

The structure, stereochemistry and absolute configuration of steviol and isosteviol were established, through a series of chemical reactions and correlations over 20 yr after the pioneering work of Bridel and Lavieille[20, 21, 22]. Structures of these and other diterpenes and diterpene glycosides are presented in Fig.6. Concurrent studies on the parent glycoside indicated that one D-glucopyranose residue, hydrolyzed under alkaline conditions yielding steviolbioside, was attached to a carboxyl group[23]while the other two were components of a sophorosyl group[24]bound to the aglycone through a β-glycosidic linkage[25].Support for the proposed stereochemistry was achieved by the synthetic transformation of steviol into stevioside[26]. Earlier, several approaches to the in vitro synthesis of steviol had been reported[27, 28, 29, and 30]**.** Recently, spectroscopic data concerning stevioside and steviolbioside were published [31].

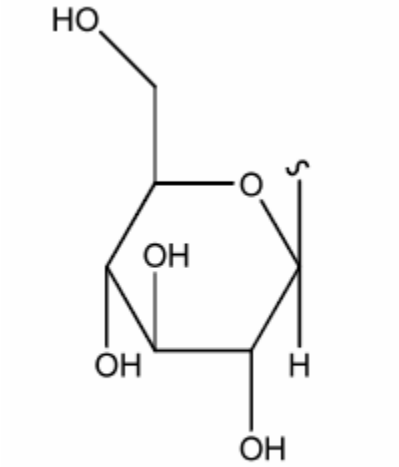
3.1.3. Structure of various glycosides:



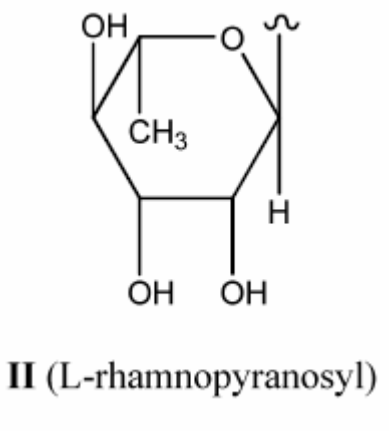
Steviol



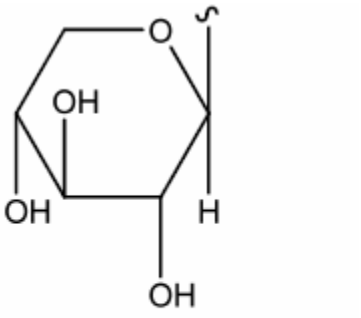
Steviol glycosides



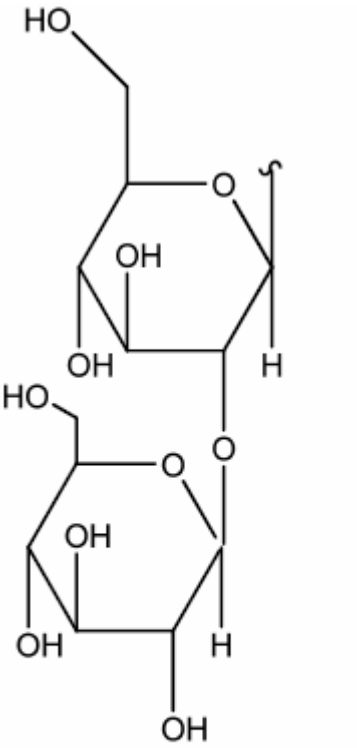
I (D-glucopyranosyl)



II (L-rhamnopyranosyl)



III (D-xylopyranosyl)



IV (β-D-glucopyranosyl-β-D-glucopyranosyl)

Figure 6: Structural formula of steviol and steviol glycosides.

Name	**R_1**	**R_2**	**R_3**	**Formula**
Steviol				$C_{20}H_{30}O_3$
Stevioside	**I**	**I**	**H**	$C_{38}H_{60}O_{18}$
Rebaudioside A	**I**	**I**	**I**	$C_{44}H_{70}O_{23}$
Rebaudioside C	**I**	**II**	**I**	$C_{44}H_{70}O_{23}$
Dulcoside A	**I**	**II**	**H**	$C_{38}H_{60}O_{17}$

Source: European Food Safety Authority (EFSA) Journal 2010, 8 *4*, 1537.

3.2. Biological Literature review:

In normal health conditions, magnesium being present in stevia promotes the secretion of insulin and the combustion of glucose and its conversion within the cells into energy available to the body. In lack of mineral; it affects both the secretion of insulin as the combustion of nutrients and the conversion of glucose into energy. The excess sugar in the blood is deposited on the walls of the blood vessels, these may swell and cause diabetes and its serious implications and it could therefore be said that,

Stevia is the plant of diabetics: Much of those affected by diabetes (more than 135 million worldwide) may benefit from the property regulating sugar levels in the blood that characterizes the stevia.

- For a 6 weeks period Goto-Kakizaki (GK) rats were given 0.025g/kg BW /day of the glycoside via the drinking water. The same amount of glucose as contained in the stevioside was given to the control group. The tail blood pressure was measured every week. At week 5 an intraarterial catheter was inserted in rats of both groups. After 6day recovery the animals were exposed to an i.v. glucose tolerance test (2.0 g/kg BW) and blood samples were drawn throughout a 180 min period. Stevioside and steviol stimulate insulin secretion from mouse islets and INS-1 cells. Stevioside and steviol seem to posses antihyperglycemic effects that

may be important in the treatment of type 2 diabetes. Stevioside and steviol seem to have an inherent advantage over the classic sulfonylurea, since the action of the diterpenes is not mediated via K^{+}_{ATP} sensitive channels. Therefore, this compound has a potential role as "anti-hyperglycemic agent" (checking the level of blood sugar) in the prevention of Type 2 diabetes mellitus (non-insulin-dependent). [32]

- The study was conducted to investigate the effects of powdered form of Stevia (*Stevia rebaudiana* Bertoni) leaves on blood glucose concentration and body weight in Streptozotocin induced diabetic rats and for its efficacy study with a patent drug, Glimepiride. The effects of powdered form of Stevia leaves was evaluated in the Streptozotocin (STZ; 55 mg/kg body weight as single intraperitoneal injection) induced diabetic rats and for this, powdered form of Stevia leaves was orally administered at three different dose rates of 150 mg/kg, 200 mg/Kg and 250 mg/Kg body weight, respectively once a day for 3 weeks. Changes in the blood glucose levels and body weights were measured and the data obtained were compared with that of Glimepiride statistically by using Student's unpaired t-test. The powdered form of Stevia leaves produced significant ($p<0.01$ or $p<0.05$) hypoglycemic effects on Streptozotocin induced diabetic rats in comparison with that of the standard drug, Glimepiride. Powdered form of Stevia leaves at a dose rate of 250 mg/Kg decreased body weight significantly ($p<0.01$ or $p<0.05$) in STZ-induced diabetic rats. From this study, it was observed that powdered form of Stevia leaves possessed both hypoglycemic and body weight reducing effects.[33]

- Stevioside and steviol seem to have an insulinotropic effect in postulated antihyperglycemic action, and both substances exert an inhibitory action on ATP phosphorylation and on NADH-oxidase activity on rat liver mitochondria, thereby causing an increase in the rate of glycolysis and suppression of gluconeogenesis[34, 35].

- Stevia extracts may benefit people at risk of diabetes, as they may help to control blood glucose levels. However, users must be careful, if they are also on anti diabetic medications. In 1986, Curi R et al, Universidad de Maringa, Brazil, demonstrated that aqueous extracts of Stevia rebaudiana leaves could increase glucose tolerance in a study of 16 healthy human subjects.[36]

- A trial in the Department of Endocrinology and Metabolism at Aarhus University Hospital, Denmark, showed that stevioside improves insulin secretion from mouse pancreas in the presence of glucose. The researchers stated, "Stevioside stimulates insulin secretion via a direct action on pancreatic beta cells." The results indicate that the compounds may have a potential role as an anti-hyperglycemic agent in the treatment of type 2 diabetes mellitus".

- In relation to diabetes, studies have shown stevia to have a re-vitalizing effect on β-cells of pancreas,[37] improve insulin sensitivity in rats,[38] and possibly even to promote additional insulin production,[39] helping to reverse diabetes and metabolic syndrome. Stevia consumed before meals significantly reduced postprandial insulin levels compared to both aspartame and sucrose.[40]

- In 2006, the World Health Organization (WHO) performed a thorough evaluation of recent experimental studies of stevioside and steviols conducted on animals and humans, and concluded "stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*." The report also found no evidence of carcinogenic activity. Furthermore, the report noted "stevioside has shown some evidence of pharmacological effects in patients with hypertension or with Diabetes mellitus type 2",[41] but concluded further study was required to determine proper dosage. The WHO's Joint Experts Committee on Food Additives has approved, based on long-term studies, an acceptable daily intake of steviol glycoside of up to 4 milligrams per kilogram of body weight.

- Two 2010 review studies found no health concerns with stevia or its sweetening extracts.[42] In addition, a 2009 review study found that stevioside and related compounds have anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory actions.[43]

4. Experimental method:

4.1. Materials and Methods:

The experiment was performed in the Department of Pharmacy, East West University Dhaka for a period of 6 months from September 2011 to March 2012.

4.1.1. Selection of patients:

20 patients aged between 40-75 years old were selected from BARDEM hospital who is suffering from diabetics mellitus (type 2).They all were controlled there food habit and some of them maintained there physical exercise. Some of them are not capable for physical exercise because of there physical illness. They take anti diabetic drug and some of them were take insulin and there were 3 patients who do not taken any medicine because they were at the starting of diabetic mellitus type 2.

4.1.2. Collection and preparation of Stevia leaves powder:

Young Stevia plants were collected from PowerAde Agro-hi-tech Limited, Uttara, and Dhaka. After that these were planted in tubs and were kept for about three months on the roof of my home. Fresh Stevia leaves were obtained from that garden. Fresh Stevia leaves collected from the garden and then sun dried. Then dried leaves were ground with the help of a Grinder machine, and from that ground sample I was distribute them to my volunteers' patients each month.

4.1.3. Dose and preparation of Stevia leaves powder:

Our daily stevia dose:

It has been estimated that sugar consumption in Japan is about 80 grams a day while in the US and Europe it is between 120 to 140 g a day .Assuming we substitute stevia for sugar, what would be our daily consumption.

For the sake of simplicity, let's say we consume about 100 grams of sugar a day. Since the sweetness of stevioside is 300 times that of sugar, the maximum daily consumption of stevia would be 100 grams divided by 300, or a third of a gram (roughly 330 mg). Actually, Chinese researchers have already estimated that the daily human consumption of stevioside would be about 2 mg per kilogram of body weight. This is a very small amount and we should keep this in mind when we evaluate the toxicity studies with Stevia done in animals. Another point to keep in mind is that most people would only partially substitute stevia for sugar and other sweeteners. Therefore, the intake of stevia on a daily basis would even be less than 330 mg.
There have been a number of studies performed in rodents and other laboratory animals to determine whether stevia has any toxicity. In many of these studies, stevia was provided in extremely high dosages, sometimes up to 5 percent of the weight of their food. Let's compare this to humans. Assuming we eat about two kilograms of food a day, and we ingest 200 mg of Stevia, the proportion of Stevia to our daily food intake would be about 0.01 percent; a very small amount, indeed.

They give progressively higher doses of the substance until a lethal dose (LD) is reached where 50 percent of the test animals die. This level is called the LD 50. Several research groups attempted to find the lethal dose of stevia .They discovered that, on average, a dose of 8,000 milligrams or more per kilogram of body weight was necessary to achieve this LD 50. In human terms, this would be equivalent to a 70 kg male ingesting more than 480,000 milligrams (or two pounds) of the extract. In most cases, a glass of water can be sweetened by less than 5 drops, an extremely minimal amount. As can be expected, no human has ever died from stevia overdose**.**

One cup sugar = about half a teaspoon of white stevia powder = about one teaspoon of liquid concentrate.
One tablespoon sugar = about six to nine drops of stevia liquid extract.
One teaspoon of sugar = about two to three drops of liquid extract.

In Bangladesh average stevioside present in green stevia leaves 8-12% in 100 gm dry leaves (international minimum acceptance level is 6-8%).Thus we gave 2gm dry green stevia powder per patient.

The recommended does for the Diabetics patient is half teaspoon (1gm) in morning and half teaspoon (1gm) in evening and they were taken the green stevia powder by boiling green stevia powder 2-3 minutes in a mug (250ml) of water like normal tea and drink when tolerable warm.

4.1.4. Determination of blood glucose level:

Biophysical examination and socio-demographic information were collected from the patients. Anthropometrics measurement for height, weight, was taken. Weight was taken with light cloths and without shoes by a modern digital scale placed on a flat surface.

Height was measured without shoes, with the subjects standing fully erect on a flat surface. Body mass index (BMI) was calculated as weight in kg/height in m^2.

At first the HbA1c report was collected from all the patients because HbA1c test shows an average of our blood glucose level over the past 10–12 weeks. Fasting blood glucose (FBG) and 2 hour after breakfast report (2ABF) were collected from all the patient at an interval of 2wk, 4wk, 6wk, 8wk, 10wk, 12wk. FBG (fasting blood glucose) from capillary whole blood was performed following the new WHO diagnostic criteria. All the subjects were then given a 75 g oral glucose solution to drink for a 2-hour post glucose challenge test. For prevalence of diabetes, FBG of ADA criteria was used. Beginning data and the last data were collected from the diagnostic center and other data ($4^{th,}$ 6^{th}, 8^{th}, and $10^{th)}$ were collected at home by the **glucometer.**

Fig: glucose meter (or **glucometer)**

4.1.5. Statistical analysis:

All data were expressed as Mean ± standard error mean (SEM) using one sample t-test. Comparison of antidiabetic activity of stevia green powder in all 20 patients was made by using one way ANOVA followed by Dunnett's multiple comparison tests. Significant level was *P < 0.05 and ** P < 0.01. Microsoft excel 2007 was used for graphical presentation. The actual percent were determined using computer software "BioStat - 2008".

4.2. Results and Discussion:

The study was conducted to investigate the effects of powdered form of stevia leaves on blood glucose concentration of randomly selected 20 type-II diabetic patients of Bangladesh. Powdered form of Stevia leaves was given by oral administration at 2 gm/day in two divided doses, half teaspoon, 1gm, in morning and half teaspoon, 1gm, in evening). Changes in the blood glucose levels were measured by glucometer and the obtained data was analysis by using one sample t-test.

4.2.1. Result:

Effects of stevia green powder on blood glucose concentration of 20 patients were comparing. On the first chart we can saw all the result of 12 weeks studies.

Fasting blood glucose mmol/L		After 2 hours of breakfast(2ABF)mmol/L
Mean ± SEM		Mean ± SEM
14.88±1.40	HbA1C% Before using Stevia green powder	
13.74±0.76	Glucose (F) Before using Stevia green powder	
14.98±0.81	Glucose (2ABF) Before using Stevia green powder	
10.73±0.56	After using Stevia green powder 2weeks	11.49±0.52
9.90±0.47	4 weeks	11.06±0.46
8.59±0.50	6 weeks	9.58±0.42
5.42±0.36	8 weeks	9.47±0.34
8.85±0.52	10 weeks	9.67±0.55

8.30±0.33	12 weeks	9.44±0.32

From this data we can saw that before use stevia the mean value of HbA1C was 14, 88% and fasting glucose value 13.74 mmol/L and after 2 hour of breakfast glucose level was 14.98 mmol/l.

At first the HbA1c report was collected from all the patients because HbA1c test shows an average of our blood glucose level over the past 10–12 weeks.

A glycosylated hemoglobin (HbA1c) test is possible because red blood cells (RBC) are continuously being made by our long bones and released into our circulation. When these cells are released, they pick up a percentage (%) of the glucose in the blood stream at that time.

Each RBC lasts about 120 days. Therefore any blood sample will have a range of cells released over the previous 120 days with different amounts of glucose attached. The

HbA1c test gives a good guide to the average.

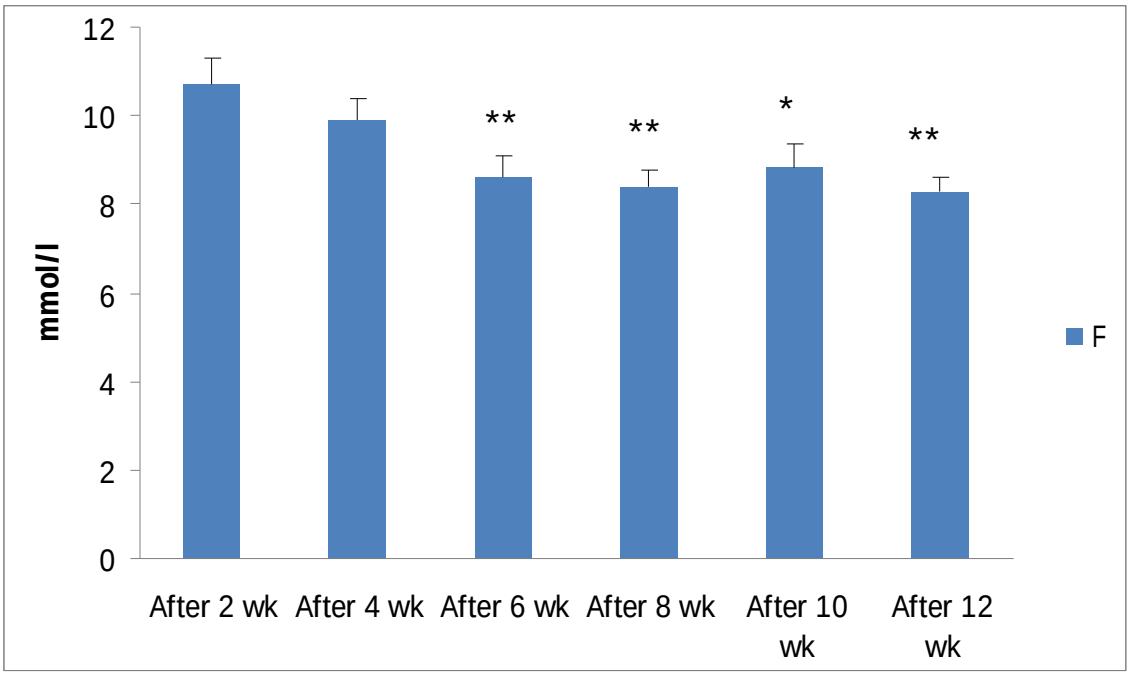
Where as the normal level of blood level is 4.4 to 6.1mmol/L .However , shortly after eating, the blood glucose level may rise, in non-diabetics, temporarily up to 7.8 mmol/L (140 mg/dL) or a bit more. The American Diabetes Association recommends a post-meal glucose level of less than 10 mmol/L.

From the data we can saw no one of the patients have controlled there diabetics, although they all have taken medicine and have controlled diet and doing there exercise.

After given stevia green powder (2gm) per day we can observe the fasting glucose level:

Fasting blood glucose after using stevia green powder	
After 2 week	10.73 mmol/L
After 4 weeks	9.9 mmol/l
After 6 weeks	8.59 mmol/l
After 8 weeks	8.42 mmol/l
After 10 weeks	8.58 mmol/l
After 12 weeks	8.3 mmol/l

Graph: 1 Effect of powdered form of Steavia green leaves on Fasting blood glucose level, after using Steavia green leaves.



Results are mean ± SEM for 20 patients. Statistical comparison was performed using ANOVA followed by Dunnett's test.

*P < 0.05 and ** P < 0.01, when compared with glucose levels of patients measured after 2 wk.

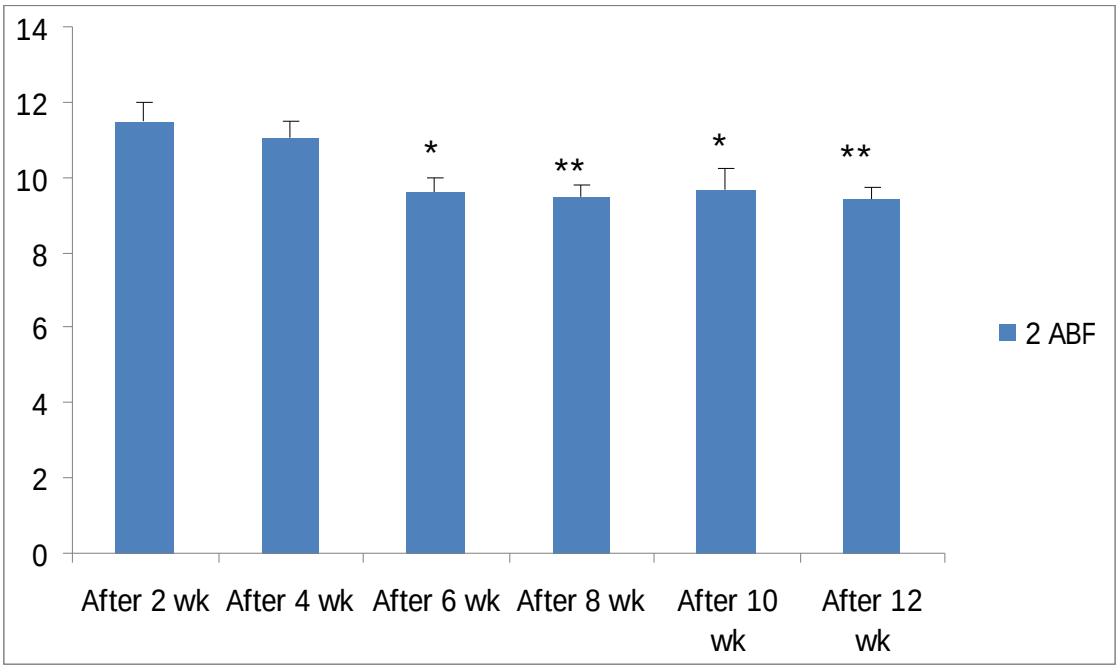
Where the fasting glucose level was 13.74 mmmol/L before using stevia green powder, we can observe the glucose level was change in a decreasing manner after using Stevia green leaves. At 2nd week the blood glucose level was 10.73mmol/L, at 4th week the blood glucose level was 9.9mmol/L, at 6th week the blood glucose level was8.89mmol/L, at 8th week the blood glucose level was 8.82mmol/L, at 10th week the blood glucose level

was 8.58mmol/L and lastly at 12th week the blood glucose level was 8.3mmol/l. The result show a very positive decreasing in blood glucose concentration of all patients. There was a constant blood glucose concentration after 6th week that was 8mmol/L.which indicate that there was no risk of hypoglycemia which is a very common problem for the diabetic patients usually when they take insulin for controlling there high blood glucose level. This problem not arises for the patients who take stevia green powder.

After given stevia green powder (2gm) per day we can observe the blood glucose level after 2 hours breakfast (2ABF):

Blood glucose level (after 2 hour breakfast) using stevia green powder	
	11.49 mmol/L 11.06 mmol/l 9.58 mmol/l 9.47 mmol/l 9.67 mmol/l 9.44 mmol/l

Graph 2: Effect of powdered form of Steavia green leaves on blood glucose level after 2 hours of breakfast (2ABF):



Results are mean ± SEM for 20 patients. Statistical comparison was performed using ANOVA followed by Dunnett's test.

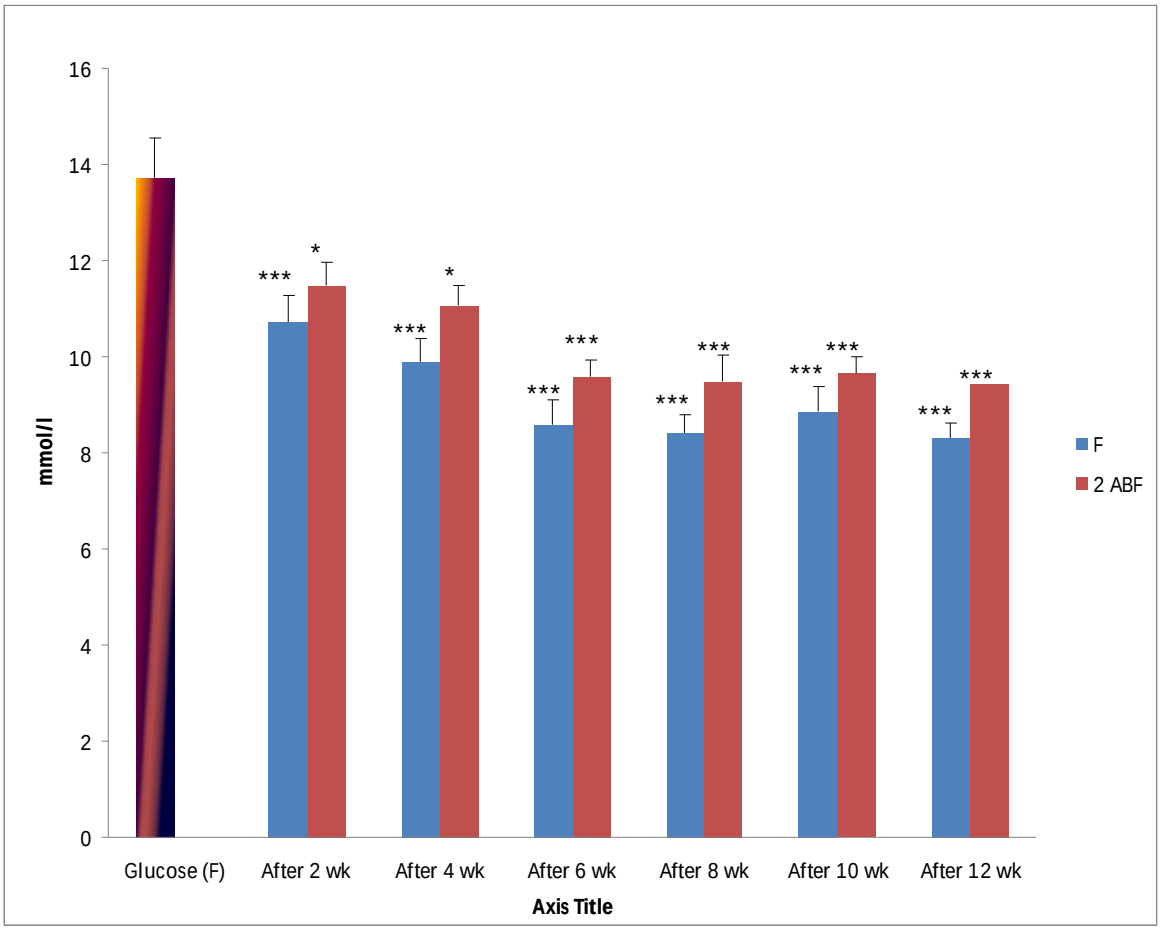
*P < 0.05 and ** P < 0.01, when compared with glucose levels of 2 ABF measured after 2 wk.

The blood glucose level (2 ABF) before using stevia green powder was14.98mmol/L.Now after using stevia green powder the blood glucose level was at 2nd week was 11.49mmol/L, at 4th week 11.06 mmol/L, at 6th week 9.58mol/L, at 8th week 9.47mmol/L, at 10th week 9.67mmol/L, and lastly at 12th week the blood glucose level was 9.44mmol/L.

Now we compare the fasting glucose level with before using stevia and after using stevia and how significant they were:

Fasting blood glucose level mmol/L		2ABF mmol/L
After 2 week	10.73	11.49
After 4 week	9.90	11.06
After 6 week	8.59	9.58
After 8 week	8.42	9.47
After 10 week	8.85	9.67
After 12week	8.3	9.44

Graph 3: Compare the fasting glucose level with before using stevia and after using stevia:



Results are mean ± SEM for 20 patients. Statistical comparison was performed using ANOVA followed by Dunnett's test.

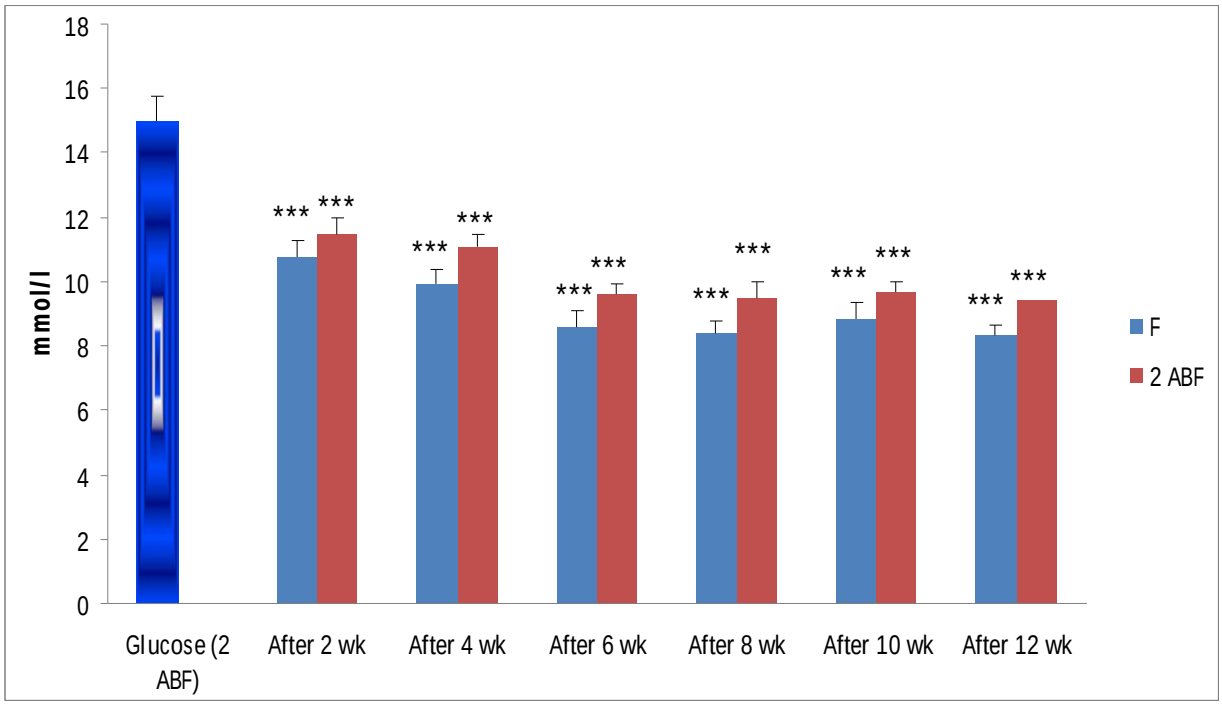
*** $P < 0.001$, when compared with Glucose (F).

The fasting blood glucose level of before using stevia green powder was 13.74mmol/L, which compare with the blood glues level of after using stevia green powder. The

powdered form of stevia leaves produce significant (*** P < 0.001) hypoglycemic effects on the blood glucose level of the patients.

Now we compare the blood glucose level after 2 hours of breakfast (2ABF) with before using stevia and after using stevia and how significant they were:

Graph 4: Compare the blood glucose level after 2 hours of breakfast (2ABF):



Results are mean ± SEM for 20 patients. Statistical comparison was performed using ANOVA followed by Dunnett's test.

*** P < 0.001, when compared with Glucose (2ABF).

The blood glucose level (after 2 hours of breakfast)of before using stevia green powder was 14.98mmol/L, which compare with the blood glues level of after using stevia green powder (2ABF). The powdered form of stevia leaves produce significant (*** P < 0.001) hypoglycemic effects on the blood glucose level of the patients.

From this data we can saw that after taking stevia green powder the result of 2week and 4^{th} week was little decreasing way that was 10.73mmol/L and 11.49mmol/L, 9.9mmol/l and 9.58mmol/L. This indicated that stevia green powder has an effect on blood glucose level.

A significant reduction ($p<0.001$) in blood glucose 8.59mmol/l and 9.47mmol/l was observed after 6^{th} week

When we collected all the sample of 12 week we observe the blood glucose level was in a very linear and static manner.

The powdered form of stevia leaves produce a significant reducing in the blood glucose concentration of diabetic patients.

4.2.2. Discussion:

There is increasing scientific validation for the use of certain traditional anti-diabetic plants and this has encouraged the search for new anti-diabetic agents. However, due to the vast number of plants and complicated purification procedures, progress has been slow. Although many plants are clamed to offer special benefits in the treatment of diabetics, few have undergone careful scientific investigation.

The study shows that the powdered forms of stevia leave have the capability to reduce the blood glucose level on diabetic patients and also there is no risk of hypoglycemic effects, because we can observe that after a time it shows a static blood glucose concentration. Our findings seem to indicate that stevia green powder may possess potential in the treatment of type II diabetes, since there have an insulin tropic effect have found that may prevent the development of hypoglycemia.

The medicinal value of this plant was trialed in Bangladesh for first time and there was also lack of related work. Further studies are needed to determine the mechanism of action of stevia green leaves, the constituent and effective dose to elucidate the blood

glucose reducing effect, and to observed side effects and adverse effects of it for taking as an anti-diabetic drug in human being.

5. Conclusion:

Diabetes mellitus is a metabolic disorder in the endocrine system. The disease is found in all parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high content of blood glucose.

The population with prediabetic conditions is always much larger than the diabetic population, but offers an opportunity for primary prevention. Studies in Finland, the United States, and China demonstrated that diabetes can be prevented in more than half of the individuals with prediabetic conditions through interventions to modify lifestyles.

In Bangladesh Stevia is an exotic plant. As the content of Stevioside in the leaves of Stevia is largely varies between 4.0% and 20% of the dry weight of the leaves depending on the cultivation and growth conditions.

In Bangladesh some tissue culture studies was done on this plant, such as *in vitro* clonal propagation of Stevia plants. Some other studies were done on animal, like diabetic rats to observe the hypoglycemic efficacy of Stevia plant.

Recently the plant *Stevia rebaudiana* is cultivated in Bangladesh. But there is no clinical report on the antidiabetic property of this plant in Bangladeshi population.

Stevia has been studies extensively around the world and there have been no reports of negative side effects. It is known to have the ability to balance the blood sugar and reduce the craving for sweets.

The US FDA itself states: "the stevioside, has reportedly been approved for use in foods in Brazil and Japan. The product is used in these countries as a table-top sweetener in virtually all food commodities and as a flavor enhancer in such products as teas."

To observe all this report, a small study on type II diabetic patient of our country was conducted to investigate the effects of powdered form of stevia leaves on blood glucose concentration of randomly selected 20 type-II diabetic patients of Bangladesh.

Powdered form of Stevia leaves was given by oral administration at 2 gm/day in two divided doses, half teaspoon, 1gm, in morning and half teaspoon, 1gm, in evening). Changes in the blood glucose levels were measured by glucometer and the obtained data was analysis by using one sample t-test.

After given stevia green powder it shows a very significant effect on the blood glucose concentration. The blood glucose concentration of before using stevia green powder was 13.74±0.76 mmol/L as fasting blood glucose level and the 2ABF blood glucose level was 14.98±0.81 mmol/L, which show that the blood glucose level was not controlled ,however patients were taking there regular medicine and doing exercise. After giving stevia green powder the blood glucose concentration was much better. After 2 weeks the concentration of fasting blood glucose was10.73±0.56 mmol/L and 2ABF was 11.49±0.52 mmol/L. After 6^{th} week the blood glucose concentration was 8.59±0.50 mmol/L as fasting blood glucose, after 8^{th} week the blood glucose concentration was 8.42±0.36 mmol/L, after 10^{th} week 8.85±0.52 mmol/L, and finally after 12 week the fasting blood glucose concentration was 8.30±0.33 mmol/L which was nearly the normal blood glucose level that is 7.8 mmol/L. The administration of powdered Stevia leaves in those 20 patients showed statistically significant reduction of blood glucose level after two hours of breakfast was 9.58±0.42 mmol/l within 6 weeks, and finally after 12 weeks blood glucose level after two hours of breakfast was 9.44±0.32mmol/L.

When all these result was compare it shows that Stevia green powder produce significant ($P < 0.05$ and $P < 0.01$) hypoglycemic effect on the patient, in comparison of that blood glucose concentration of before using stevia green powder.

Thus, the powdered Stevia leaves produced significant hypoglycemic effects on blood glucose concentration when compare with the blood glucose level of before using stevia green powder of 20 type-II diabetic patients. From this study, it was observed that the

compound *Stevia reabaudiana* green leaf powder may have a potential role as antihyperglycemic agents in the treatment of type-II diabetes mellitus in Bangladeshi population.

During the study some other positive sign from the patient about stevia green leaves. When the last blood glucose report was collected from the patients they reported that, they feel very good after taking stevia green powder, in last three month they don't suffer from any hypoglycemic as well as hyperglycemic shock. They have no tooth problems, some of them were relief from there excess urination problems which occur due to there prosted gland enlargement. They were very happy to make some sweet food from stevia green powder.

During this study there was some lacking that should be clear,

- Study should be done in six mounts, thus the result will be more clear and effective.
- Some negative controlled patients will be needed for verify the current results.

Stevia has granted attention with the rise in demand for low carbohydrate, low-sugar food alternatives. Stevia has desirable effect on blood glucose, even enhancing glucose tolerance, and therefore it is attractive as a natural sweetener to diabetics and others on carbohydrate-controlled diets.

It has been proven that Stevia leaves if consumed regularly have great potential in reducing the dependence on the oral and injectable insulin. Regular intake of Stevia leaves induces the beta cells of pancreas to produce more insulin. The interesting property of stevia leaves opens up number of possibilities for Ayurvedic and herbal drug manufactures to –

- Improve the existing formulation targeted at controlling diabetes.
- To develop new formulations to tackle this severe problem of type I and II diabetes in Asian population wind.

Stevia is likely to become a major source of commercial sweetener for the growing natural food market in the future. At present understanding the biology of the stevia plants and biochemistry of the sweet glycosides are prerequisites for conversion of stevia to a modern crop.

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