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ANTIOBESITY REPORT OF A SWEET SUPER LEAVES, *STEVIA REBAUDIANA* (BERT.) IN MICE MODEL

Shaheen SM[1*]; Azad AKM[1], Ferdous W.[1] and Uddin Jashim M.[2]

[1]Daffodil International University, Daffodil Tower (DT)-054/2 Sobahanbag, Mirpur Road Dhanmondi, Dhaka-1207, Bangladesh.Tel:+880248111639, Fax+88029131947

[2]Department of Biochemistry, Vanderbilt University Medical Center, 850 RRB, TN, USA

E-mail address: sharif.ph@diu.edu.bd

Abstract

The plant "*Stevia rebaudiana* (Bert.)" is a glorious sweet plant of almost no calorie and famous as natural sweetener. However, till now many phytochemical and pathological screening as well as pharmacological screenings are yet to be done. Here various pathological and phytochemical as well as pharmacological screening were carried out. Phytochemical screening revealed that alkaloids, glycoside, carbohydrates, protein, tannin, saponin, anthraquinone and flavonoids are present in the leaf extract of "*Stevia rebaudiana* (Bert.)". The constituents of stevia usually vary from zone to zone, depending on the weather conditions of respective area. The results of lipid profile screening showed mild to moderate antihyperlipidemic activity in TC, LDL, HDL, VLDL & TG. The "*Stevia rebaudiana*" extract showed a significant lipid lowering activity in soya oil and cow's fat induced albino mice ($P \leq 0.05$). Stevia showed a moderate response against obesity, showing anti-lipase activity. The study is carried out with an orlistat standard where orlistat had 42% lipase inhibitory property whereas stevia had 31.4% lipase inhibitory activity. The green house originated stevia showed all above activities. Collectively we may conclude that stevia can be a good substitute of natural sugar in obese patients, which may change life-style of the patient complaints.

Keywords: *Stevia rebaudiana, phytochemical screening, Antidiabetic activity, Antihyperlipidemic activity, Anti-obesity activity*.

Introduction

The practice of Traditional medicine is deeply rooted in the cultural heritage of Bangladesh and constitutes an integral part of the culture of the people of this country. Different forms of traditional medicines have been used in this country as an essential means of treatment of diseases and management of various health problems from time immemorial. The practice of traditional medicine in this country has flourished tremendously in the recent years along with that of modern medicine. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country, particularly in the rural and semi-urban areas, still prefer to use traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighborhood. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs [1].

The most common reasons for using traditional medicine are that it is more affordable, more closely corresponds to the patient's ideology, allays concerns about the adverse effects of chemical (synthetic) medicines, satisfies a desire for more personalized health care, and allows greater public access to health information. The major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening, conditions. However, usage of traditional remedies increases when conventional medicine is ineffective in the treatment of disease, such as in advanced cancer and in the face of new infectious diseases. Furthermore, traditional medicines are widely perceived as natural and safe, that is, not toxic. This is not necessarily true, especially when herbs are taken with prescription drugs, over-the-counter medications, or other herbs, as is very common [2].
Stevia leaves are said to be from 30 to 300 times sweeter than sugar. It is touted as a natural alternative to artificial sweeteners. To date, chemical analysis and studies show that the leaf adds no calories, has no harmful side effects and is more palatable with less aftertaste than any artificial, chemical sweetener to date. Stevia has been in wide use in South America for centuries, and in Japan since the government banned the use of artificial sweeteners [3]. Besides these it has got the potentials of the followings published elsewhere [4]: a) The stevia leaves contain potassium,zinc, magnesium and vitamin B3; b) Based on the stevia nutrition facts, it is the 10.77% fiber which helps in curing constipation and promotes proper digestion; c) One of the benefits of stevia is that it contains a 702 ppm level of iron which can stimulate the production of hemoglobin.
Stevia rebaudiana Bertoni is a versatile herb with incredible sweetness that is gaining very high popularity amongst all type of sweetener users as most ideal substitute for sugar. It produces sweet steviol glycosides. It is a high demanding antidiabetic medicinal plant belonging to Asteraceae family. It is perennial and endemic, medicinal herb. It is also called as honey plant due to its sweetness. Thus the present study is a preliminary attempt to identify some of Ethnopharmacological activities. Hopefully this will lead to new information on this plant application and new perspective on the potential use of *Stevia.*

The sweet herb of Paraguay, *Stevia rebaudiana* Bert. produces, in its leaves, just such an alternative with the added advantage that stevia sweeteners are natural plant products. In addition, the sweet steviol glycosides have functional and sensory properties superior to those of many other high-potency sweeteners. Stevia is likely to become a major source of high-potency sweetener for the growing natural food market in the future. The task at hand is to convert stevia from a wild plant to a modern crop well suited to efficient mechanized production. For Canada, the necessary steps are the development of a 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.

Understanding the biology of the stevia plant and the chemistry and biochemistry of the sweet glycosides are prerequisites for conversion of stevia to a modern crop [5]. *Stevia rebaudiana* is an herbaceous perennial shrub in the family Asteraceae

(the composite, or daisy, family) that is native to northeastern Paraguay and adjacent Brazil and Argentina, China, Canada, Paraguay, and Indonesia. In its native state, *Stevia rebaudiana* grows on the edges of marshes or in grassland communities on soils with a shallow water table. The sweetening power of its leaves have long been known to the local Guaraní Indians and others (Lewis 1992). It is indigenous to the Rio Monday Valley of the Amambay moutain region at altitudes between 200 and 500 m. The climate in this area is semi-humid subtropical, with temperatures ranging from -6 to 43 C, with an average of 23 C, and annual rainfall ranging from 1500 to 1800 mm. In 1943, the first seeds were exported to the United Kingdom, but the plants were not successfully brought into cultivation. In 1968, *Stevia rebaudiana* was exported to Japan and from there awareness of and cultivation of the plant spread throughout the world. It has now been introduced to many countries, including Brazil, Korea, Mexico, the United States of America, Indonesia, Tanzania, Canada, and India. Individuals of this species are self-incompatible and probably insect-pollinated. This is a short-day plant that flowers from January to March in the southern hemisphere and from September to December [6].

However, the constituents vary from zone to zone and biological evaluation is needed to compare. Here we have used stevia of our green house condition and screened for a lot of studies. In this case we focused on obesity because it provides almost no calories. Obesity is a disaster of both the developed and developing countries.

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kg) divided by the square of his or her height (in m). A person with a BMI of 30 or more is generally considered obese. A person with a BMI equal to or more than 25 is considered overweight. Overweight and obesity are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings [7]. Though stevia provides almost no calorie however there might be a link up with the weight loss or gain needed to be investigated. Here we have evaluated lipid profiles like TG, Cholesterol, LDL, VLDL and lipase activity on a few of obese mice treated with stevia.

Materials and Methods

Experimental animals

For the study, 30 swiss albino mice of both sexes took from the Animal House of the Department of Pharmacy, Jahangirnagar University. These animals were apparently healthy and weighed 12-15 g.

The animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. All of the mice were kept in steel cages having dimensions of 30x20x13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animals was done ad libitum, along with drinking water and maintained at natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka). All experiments on mice were carried out in absolute compliance with the ethical guide for care and use of laboratory animals.

Preparation of extract materials

About 200 gm of powdered leave material was taken in clean, round bottomed flask (1 liters) and stored at room temperature in 500ml of ethanol for 10 days with occasional shaking for better extraction. The whole mixture was then filtered through cotton. After filtration the filtrate was concentrated at 40°C with a rotary evaporation. The concentrated extract was then preserved in refrigerator. The weight of the crude ethanolic extract of leaves was obtained 4.43gm.

Experimental design

24 swiss albino mice weighing 22-25 g were used for the study while 12 male albino mice and 12 female albino mice were used. After acclimatization, the mice were divided into 4 group of 6 mice. Among them, positive control group and normal group were not treated by drug or standard. After induction of hyperlipidemia, 20mg/kg b.w. ethanol extract of *Stevia rebaudiana* and 20mg/kg b.w. atorvastatin were administered orally daily for 21 days. Thereafter

LDL, VLDL, TG and total cholesterol tests were monitored [8].

Lipase Preparation

The enzyme solution was prepared immediately before using the following method described by Bustanji with some modifications [9]. Crude porcine pancreatic lipase type II was suspended in 20 mM tris-HCl buffer pH 8 to give a concentration of 10 mg/ml. The suspension was mixed using a stirrer for 15 min and centrifuged at 1500 g for 10 min and the clear supernatant was recovered.

Lipase Inhibition Reaction

Porcine pancreatic lipase (PPL, type II) activity was measured using p-nitrophenyl butyrate (p-NPB) as a substrate. The method used for measuring the pancreatic lipase activity was modified from that previously described by Kim, et al. and Zheng, et al.[10], [11]. PPL stock solutions (1 mg/mL) were prepared in a 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions were stored at –20 °C. To determine the lipase inhibitory activity, the extracts (final concentrations 100, 50, 25, 10, 5, 2.5, 1.25 µg/mL) or Orlistat (at same concentrations) as a positive control were pre-incubated with PPL for 1 h in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30 °C before assaying the PPL activity. The reaction was then started by adding 0.1 µL NPB as a substrate, all in a final volume of 100 µL. After incubation at 30 °C for 5 min, the amount of p-nitrophenol released in thereaction was measured at 405 nm using a UV-Visible spectrophotometer (BioTek Synergy HT, Winooski, VT, USA). The activity of the negative control was also examined with and without an inhibitor. The inhibitory activity (I) was calculated according to the following formula [9]: Lipase In% = (ΔControl-ΔSample)/ ΔControl x 100, where ΔControl = Control -blank, Δsample = Test sample – blank.
Inhibition of the lipase activity was expressed as the percentage decrease in the activity when PPL was incubated with the test compounds.

Results and Discussion

Phytochemical groups screening
After completing wide range of chemical test for the identification of major classes of therapeutically important compounds, carbohydrate, glycoside, flavonoid, tanin and alkaloids are present in the leaf extract of "*Stevia rebaudiana* (Bert.)". The following table will give us a broad idea about phytochemical groups present in these plants (Table 1). The data showed the plenty carbohydrate present which is a controversial matter of stevia providing almost zero calorie. However, the effect of other components found in this plant might have sugar catabolic effect providing no effect, which needs to be further investigation. Plenty alkaloids, flavonoids, saponin and terpenoids have numerous medicinal values of different pharmacological effects those should under consideration of future research.

Lipid profile studies treated with stevia on mice model

From the enzymatic analysis of lipid profile after 21 days trial, we found that Group A (normal control) TC, HDL, LDL, VLDL & TG value was 84.12 ± 4.80, 40.28 ± 2.30, 26.32 ± 3.10, 17.52 ± 1.60 & 87.62 ± 7.26 mg/dl respectively, Group B (positive control) TC, HDL, LDL, VLDL & TG value was 146.82 ± 1.40, 25.00 ± 1.86, 26.32 ± 3.10, 29.68 ± 1.40 & 148.42 ± 5.06 mg/dl respectively, Group C (Standard with metformin) TC, HDL, LDL, VLDL & TG value was 118.62 ± 4.22, 32.20 ± 2.12, 62.28 ± 4.28, 24.14 ± 1.40 & 120.68 ± 4.76 mg/dl respectively and Group D (treatment with stevia extract) TC, HDL, LDL, VLDL & TG value was 104.46 ± 5.18, 34.98 ± 2.10, 49.06 ± 3.82, 20.42 ± 1.10 & 102.12 ± 4.38 mg/dl respectively that shows a significantly improved the lipid profile level (Figure 1). So, demonstrate that ethanolic extract of *Stevia rebaudiana* possesses significant antihyperlipidemic activity.

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Table 1: List of phytochemical groups found in *Stevia rebaudiana*

Group name	Name of the Test	Result
Carbohydrate	Molisch Test	+++
	Fehling's Test	+++
Tannins	10% PotassiumDichromate	-
	5% Ferric Chloride	++
	1% Lead Acetate	+
Alkaloids	Mayer's Test	++
	Dragendroff's Test	++ +
	Hager's Test	+
Saponins	Test for Saponins	+ +
Anthraquinone	Test for Coumarin	+
Flavonoids	Test for Flavonoids	+ +
Glycoside	Baljet's test	++
	Liebermann's test	++
	Borntrager's test	++
Proteins	Biurets's Test	+
Terpenoids (Steroids)	Salkowski test	++

[Symbol -= Absent, += Present, ++= Moderately Present, +++= Strongly Present]

Figure 1: Lipid profile parameters using atorvastatin and stevia respectivly.

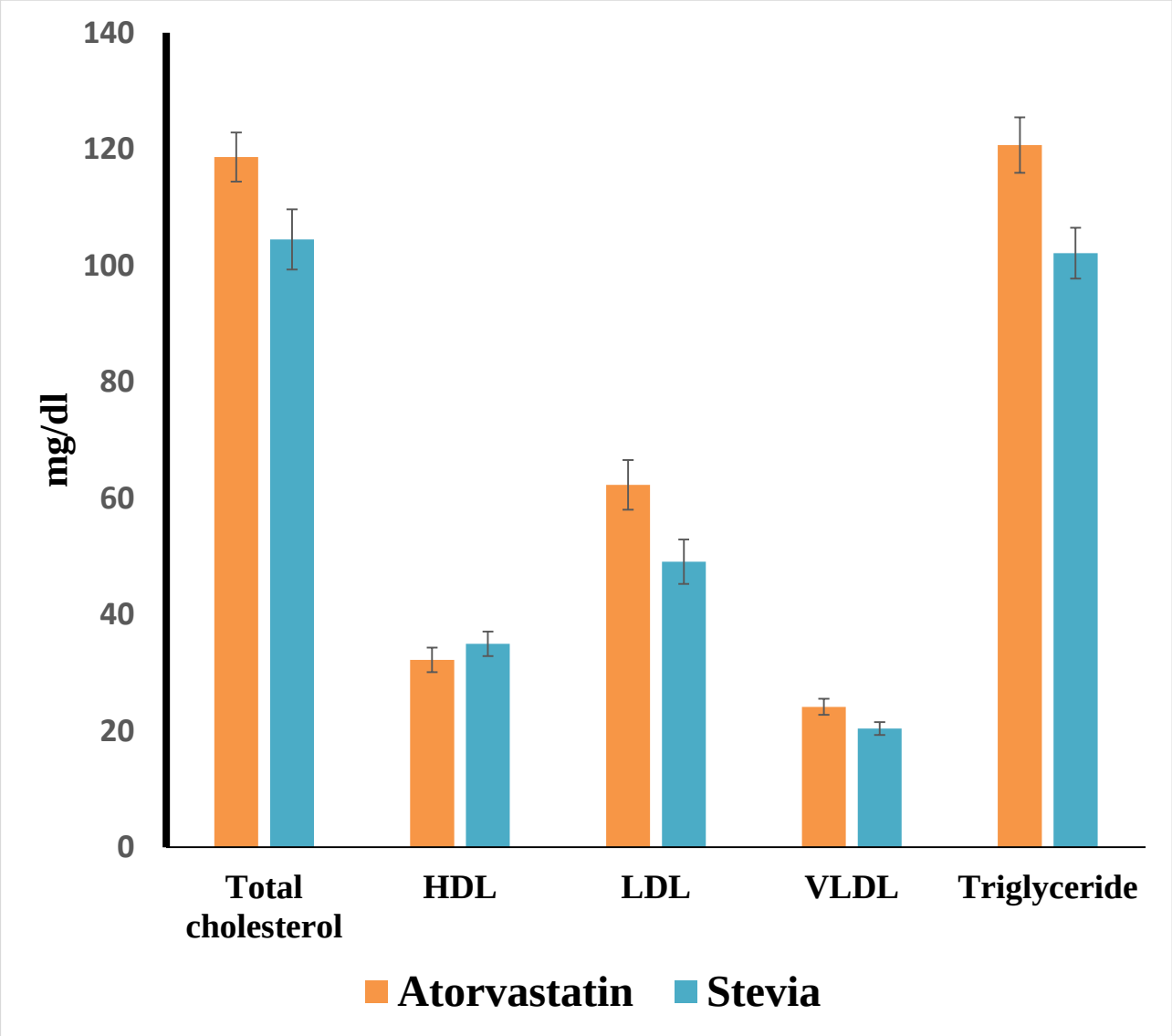

Figure 2: Lipase activity inhibition using a standard orlistat and stevia, respectively.