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Immune up regulatory response of a non-caloric natural sweetener, stevioside

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Abstract

Immunomodulation is a process, which alters the immune system of an organism by interfering with its functions. This interference results in either immunostimulation or immunosuppression. An immunomodulator is any substance that helps to regulate the immune system. This "regulation" is a normalization process, so that an immunomodulator helps to optimise immune response. Immunomodulators are becoming very popular in the worldwide natural health industry as these do not tend to boost immunity, but to normalize it. Keeping this in view, major efforts have to be directed to modulate the immune responses, to permit effective treatment of various ailments associated with immune system and thus the development of a safe and effective immunomodulator for clinical us.

Leaves of *Stevia rebaudiana* are a source of several sweet glycosides of steviol. The major glycoside, stevioside, diterpenoid glycoside—is used in oriental countries as a food sweetener. Its medical use is also reported as a heart tonic. Besides, it is used against obesity, hypertension, and stomach burn and to lower uric acid levels. Here in this study, stevioside was tested for its immunomodulatory activity on different parameters of the immune system at three different doses (6.25, 12.5 and 25 mg/kg p.o.) on normal as well as cyclophosphamide treated mice. Stevioside was found effective in increasing phagocytic activity, haemagglutination antibody titre and delayed type hypersensitivity. In parallel, stevioside substantially increase proliferation in the LPS and Con A stimulated B and T cells, respectively. Present study, therefore, reveals that the drug holds promise as immunomodulating agent, which acts by stimulating both humoral as well as cellular immunity and phagocytic function.

Keywords: Stevioside; Immunomodulatory activity; Humoral response; Cellular immunity; Phagocytosis

1. Introduction

Leaves of *Stevia rebaudiana* are a source of several sweet glycosides of steviol [1]. The major glycoside, stevioside, diterpenoid glycoside—is used in oriental countries as a food sweetener. Other major glycosides named rebaudioside, which is sweeter and more delicious than stevioside, is utilised in beverages. To improve the sweetness and the taste, modifications of sugar moieties of both the glycosides were performed by enzymatic glycosylations and/or enzymatic trimming.

Stevioside (Fig. 1) is a sweet-tasting glycoside occurring abundantly in the leaves of *S. rebaudiana* (Compositae). It has been used popularly in Japan and Brazil as a sugar substitute for decades. Previous study has shown that it lowered blood pressure in spontaneously hypertensive rats (SHRs) when administered intravenously. This study shows that intraperitoneal

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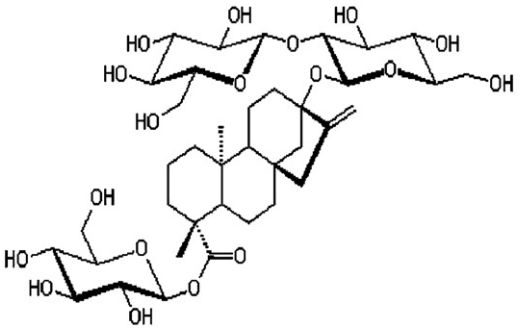

Fig. 1. Structure of stevioside.

injection of stevioside 25 mg/kg also has antihypertensive effect in SHRs [2].

For hundreds of years, indigenous people (Guaranis) native to Paraguay and Brazil have used its leaves as a sweetener, particularly to sweeten their tea (mate), which is very consumed among these tribes. Its medical use is also reported as a heart tonic. Besides, it is used against obesity, hypertension, and stomach burn and to lower uric acid levels [3].

The first written article about it dates back to 1900. In 1931, however, it has been found that the glycosides are the responsible constituents for the sweetening properties of its leaves [4]. Post phytochemical analyses of the results have recorded the presence of 5–10% of stevioside, 2–4% of rebaudioside and dulco-sides. Stevioside is the sweetest and its sweetening power was shown to have been 300 times greater than sacarosis and accounted for 18% of the leaf total composition.

Today, Stevia sweetener has been traded almost all over the world and has been used to sweeten hundreds of diabetic products, particularly soft drinks. The Japanese are considered to be the greatest consumers. In addition to being a non-caloric sweetener, this plant has been reported to be hyploglycemiant, hypo-tensor, diuretic and cardiotonic. In Brazil, it has been successfully used as the most suitable sweetener for diabetic people.

A great deal of clinical studies has validated such uses even in the USA, where its employment is forbidden due to the pressure and the lobbying led by the powerful industry of artificial sweeteners [5]. The present investigation was aimed to study the immunomodulatory activity of stevioside using reported methods in order to justify the drug as an immunomodulator.

2. Materials and methods

2.1. Animals

Balb/c mice of either sex, 10–12 weeks old, weighing 20–25 gm were randomly distributed in groups as per experimental protocols ($n = 6$). Animals were housed and maintained following standard guidelines (CPCSEA, 2003). The study protocol was approved by Institutional Animal Ethics Committee.

2.2. Test sample

Stevioside was prepared as freshly homogenized suspension in 1% (w/v) gum acacia and administered orally daily once a day for the duration of the experiment. The control animals were given an equivalent volume of gum acacia vehicle. Cyclophosphamide (200 mg/kg p.o.) was used to induce immunosuppression 2 days prior to immunization with SRBC. LPS and Con A were used as standard mitogens for *in vitro* lymphocyte proliferation.

2.3. Antigen

Sheep red blood cells (SRBCs) were used as antigen. Fresh sheep blood was collected aseptically from the jugular vein of the sheep in sterile cold Alsever's solution. Blood was washed three times with pyrogen free sterile normal saline (0.9% NaCl w/v) and 0.2 ml of 5×10^9 SRBCs/ml were used for immunization. Animals were challenged on 6th day from the day of immunization [6].

2.4. Effect on general behaviour and acute toxicity

The acute oral toxicity studies were carried out following OECD guidelines. Three different doses 6.25, 12.5 and 25 mg/kg body weight of test drug were administered orally to the groups of six mice. The animals were observed for 14 days for any change in gross behavior and mortality.

2.5. Humoral antibody (HA) titre

The method described by Nelson and Midenhall was adopted [7]. Humoral immunity was determined in normal as well as immunosuppressed mice. For immunosuppression a single dose of cyclophosphamide (200 mg/kg, p.o.) was administered on 2-day prior to immunization (day 0). Animals were divided into groups of six mice each. The control group received 1% gum acacia solution only as vehicle; while animals in the treatment groups were given the test drugs (6.25, 12.5 and 25 mg/kg, p.o.) daily for 7 days. The animals were immunized by injecting 0.2 ml of 5×10^9 SRBC/ml suspension i.p. on 0 day. Blood samples were collected from individual animal by retro-orbital plexus on 7th day to obtain serum. Antibody levels were determined